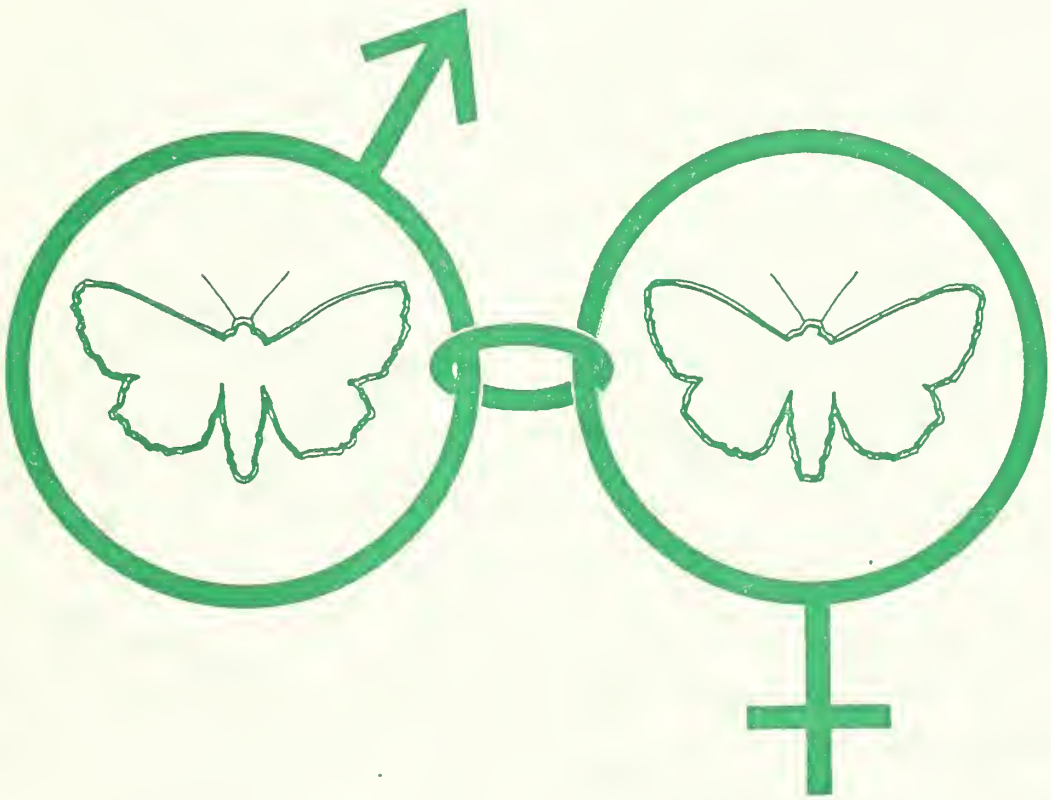
Insect Attractants, Behavior, and Basic Biology Research Laboratory

Gainesville, Florida

PROGRESS REPORT —— 1994



AGRICULTURAL RESEARCH SERVICE
US DEPARTMENT OF AGRICULTURE
SOUTH ATLANTIC AREA

FOR OFFICIAL USE ONLY

FOR OFFICIAL USE ONLY

PROGRESS REPORT – 1994

INSECT ATTRACTANTS,

BEHAVIOR, AND BASIC BIOLOGY

RESEARCH LABORATORY

AGRICULTURAL RESEARCH SERVICE

U. S. DEPARTMENT OF AGRICULTURE

P. O. Box 14565, Gainesville, Florida 32604

904-374-5701

FAX # 904-374-5781

This report includes results of research in progress.
It is not intended for publication, and should not be
referred to in literature citations

June 5, 1995

The research mission of the Insect Attractants, Behavior and Basic Biology Research Laboratory is based on the premise that biologically rational, environmentally safe pest control requires basic research on unique vulnerabilities in the insect life cycle. This report is provided as a courtesy to our cooperators and other interested scientists. Recently published articles may be requested by writing the laboratory office.

In 1994 Drs. R. T. Arbogast and D. K. Weaver transferred to our laboratory to strengthen our research program on detection and protection research with stored product insects. There are now six permanent staff scientists and three post-doctoral positions devoted to research on stored product entomology.

During 1994 we continued research on several Pilot Tests: "Assessment of Gibberellic Acid as a New Tool for Management of Tephritid Fruit Flies" directed by Dr. Patrick Greany; "Control of *Heliothis/Helicoverpa* and Army worms in Cotton with Semiochemicals", directed by Dr. Everett Mitchell; and "Integration of Mating, Disruption and Parasitoids to Control Diamondback Moth in Cruciferous Vegetables", directed by Dr. Everett Mitchell. In addition we were active in developing Cooperative Research and Development Agreements (CRADAs) in the areas of biological control, acoustical detection of hidden infestations and semiochemical monitoring systems. These Pilot Tests and CRADAs provide opportunities for us to develop to a practical level some of the key technologies that have been discovered at this laboratory.

We gratefully acknowledge the United States-Israel Binational Agricultural Research and Development Fund, The California Department of Food and Agriculture, and the USDA National Research Initiative Competitive Grants Program for providing extramural support for our research programs during the past year. We are appreciative of the cooperation and resources provided to us through our interactions with industry including Biocontrol Limited, Consep Membrane, Inc., Defense Research Technolgies, Inc., Ecogen, Inc., EPCOT/The Land, Predation, Inc., the Rohm and Haas Co. and the Shin-Etsu Chemical Company. We also extend our thanks to the departments of Entomology and Nematology, and Agricultural Engineering at the University of Florida, the Division of Plant Industry of the State of Florida, and to our many other cooperators.

Herbert Oberlander

Herbert Oberlander
Laboratory Director

PERSONNEL AND UNITS OF THE INSECT ATTRACTANTS, BEHAVIOR, AND BASIC BIOLOGY RESEARCH LABORATORY

P. O. BOX 14565
GAINESVILLE, FL 32604
(904) 374-5701

****

H. Oberlander, Director
P. Egger, Secretary
R. Bradley, Student Assistant

BEHAVIOR AND BIOPHYSICS

E. R. Mitchell, Research Leader
- V. Malcolm, Secretary
- W. W. Copeland, Biological Technician
- J. Leach, Biological Technician
- J. Gillett, Biological Aid
- R. Furlong, Biological Technician
- E. Rosa, Laboratory Technician
- E. Lanehart, Laboratory Technician
- J. Okine, Entomologist
- G. Hu, Entomologist
- N. Doran, Laboratory Technician
- I. Smith, Laboratory Technician
- K. Gallagher, Student Aid

P. J. Landolt, Research Entomologist
- T. Macom, Laboratory Technician
- S. Lovvorn, Student Aid
- O. Molina, Laboratory Technician
- P. Ponce, Graduate Student
- A. Harari, Visiting Scientist

M. S. Mayer, Research Entomologist
- C. A. Litzkow, Electronics Engineer
- J. E. Sharp, Biological Technician

J. Sivinski, Research Entomologist
- G. Posey, Biological Technician
- A. Eitam, Graduate Student
- K. Vulinec, Graduate Student

BIOREGULATION AND MOLECULAR GENETICS

H. Oberlander, Research Leader
- P. Egger, Secretary
- C. E. Leach, Entomologist

R. T. Arbogast, Research Entomologist
D. Weaver, Research Entomologist
S. M. Ferkovich, Research Entomologist
C. R. Dillard, Biological Technician
P. Gupta, Research Associate
B. Gough, Student Aid
P. D. Greany, Research Entomologist
M. Pendland, Student Aid
J. Venn, Biological Technician
A. M. Handler, Research Geneticist
S. Gomez, Research Associate
G. P. Whitmer, Biological Technician
R. W. Mankin, Research Entomologist
E. Foreman, Computer Programmer
B. A. Weaver, Biological Technician
S. G. Miller, Research Geneticist
R. D. Miller, Biological Technician
P. D. Shirk, Research Physiologist
M. Hapner, Biological Technician
J. Goodrich, Student Aid
R. Broza, Laboratory Technician
D. Shuman, Research Electrical Engineer
R. Johnson, Computer Programmer
D. L. Silhacek, Research Chemist
C. L. Murphy, Biological Technician
S. Dyby, Research Associate
S. Parisek, Student Aid

CHEMISTRY

J. H. Tumlinson, Research Leader
P. Zelonka, Secretary
H. Alborn, Visiting Scientist
Y. Gazit, Visiting Scientist
G. Makranczy, Visiting Scientist
U. Röse, Visiting Graduate Student
M. M. Brennan, Physical Science Technician
J. Lockerman, Student Aid
A. Reisinger, Student Aid
P. E. A. Teal, Research Physiologist
J. Meredith, Research Affiliate
R. Abernathy, Graduate Student
R. E. Doolittle, Research Chemist
I. Smith, Laboratory Technician
R. R. Heath, Research Chemist
N. Epsky, Research Associate
B. D. Dueben, Physical Science Technician

A. T. Proveaux, Physical Science Technician
C. P. Powell, Chemist
J. Baum, Student Aid
Y. Cardoza, Student Aid
J. Howell, Student Aid
E. Gunter, Student Aid
E. Paulsen, Student Aid

INSECT REARING STAFF

E. R. Mitchell, Supervisor (Field Insects)
F. Adams, Biological Technician
T. Nguyen, Laboratory Technician
C. Green, Laboratory Technician
G. Encabo, Laboratory Technician
D. L. Silhacek, Supervisor (Stored Prod. Insects)
L. Wojcik, Biological Technician
N. Lowman, Laboratory Technician

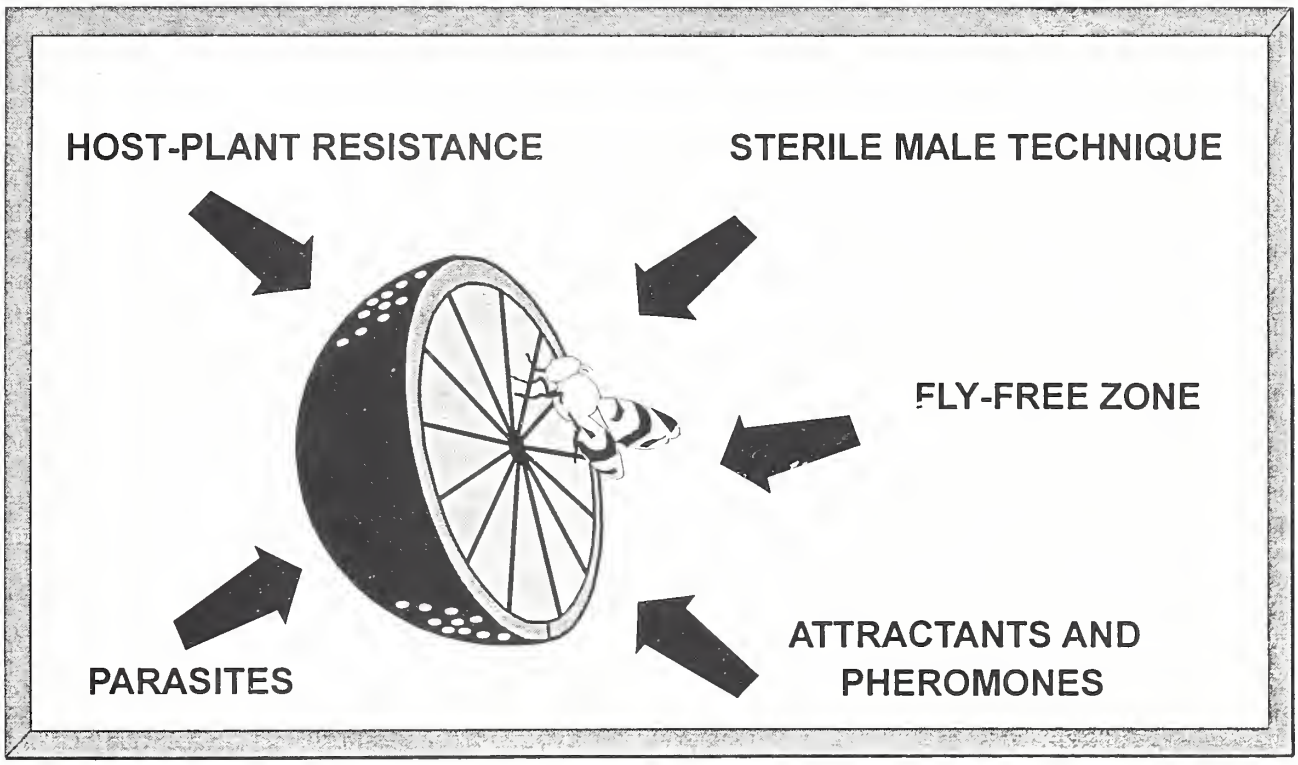
TABLE OF CONTENTS

CITRUS

FIELD CROPS

STORED PRODUCTS

CITRUS


HOST-PLANT RESISTANCE
STERILE MALE TECHNIQUE
FLY-FREE ZONE
PARASITES
ATTRACTANTS AND
PHEROMONES

SPATIAL ANALYSIS OF TRAP DATA FOR MEDITERRANEAN FRUIT FLY CONTROL PROGRAMS

R. Abernathy, N.D. Epsky, and R.R. Heath

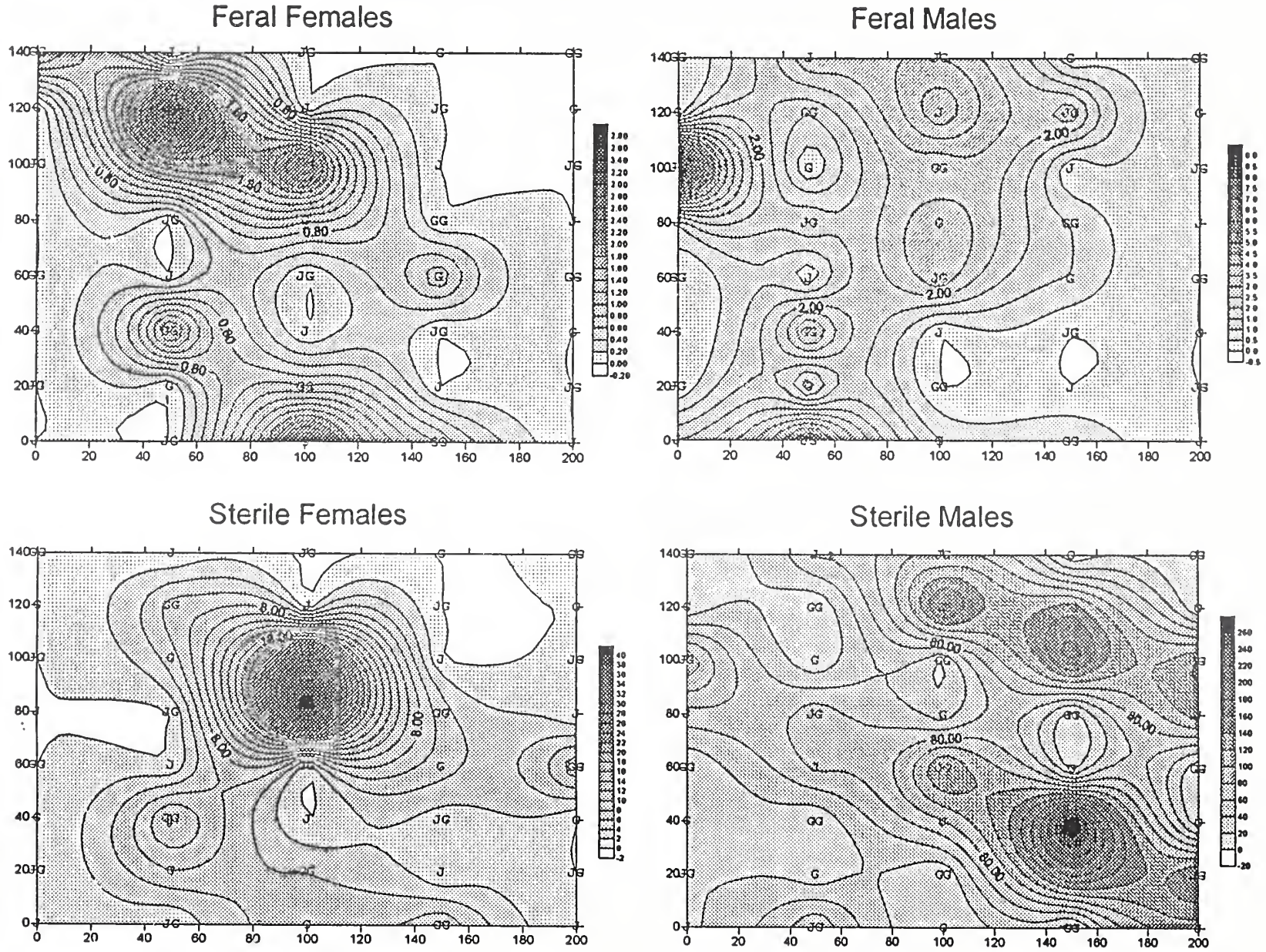
Objective: To develop new techniques for the interpretation of trapping data for fruit flies.

Methods: Standard techniques for the analysis of data from field experiments tend to lose all spatial information. Because of this, much of the ecological information is lost as well. It is therefore desirable to find techniques of statistical analysis that, while maintaining the inferences gained by standard statistical techniques, also respect the underlying spatial processes. Therefore, we are examining the use of spatial statistics and specifically to the spatial inference technique known as kriging. Kriging was originally developed by geostatisticians for use in the mining industry, but it is now finding a use in various areas of ecology. Kriging is a mathematical technique that generates a spatial distribution based on sampled spatial data. The theoretical distribution is produced using variances as a function of distance from the sampled data. We have used these spatial techniques to gain new information on medfly population distributions.

Results: Figure 1 shows contour plots of population distributions inferred by kriging from trapping data for a one week period in a coffee field in Guatemala. The data was obtained for both feral and sterile, male and female flies using two different trap designs. The two trap types were the dry trap baited with a synthetic food-based attractant and the standard trimedlure-baited Jackson trap. Traps were placed in the field in a 20 m by 50 m grid, with alternating trap types. Figure 1 shows the differences among the distributions for the different categories of insects as indicated from trapping data. If standard statistical techniques had been used these spatial differences would not have been apparent.

Plans: Spatial information could provide significant input on the efficacy of fruit fly control and eradication approaches. To further enhance the effectiveness of spatial techniques towards the accomplishment of this goal, ecological data will be combined with the population sample data for the determination of the population distribution. We will use universal kriging for this purpose. We are currently investigating the process of developing universal kriging models for medfly populations using geographical information systems (GIS) software to obtain and analyze the ecological data.

Figure 1. Trap Location and Distribution of Medflies Coatepeque, Guatemala



THE BIOGEOGRAPHY OF THREE SPECIES OF CARIBBEAN FRUIT FLY PARASITOIDS IN FLORIDA

A. Eitam and J.M. Sivinski

Objective: To characterize the range and habitat preferences of the three major species of braconid parasitoids of the Caribbean fruit fly in Florida. This would allow tailoring the composition of augmented parasitoid releases to specific areas.

Methods: In cooperation with USDA-APHIS and the Florida Department of Agriculture monthly systematic samples of Caribbean fruit fly host fruit are being made over central and southern Florida. Fruit are held in the laboratory and the emerging insects identified.

Results: Preliminary results indicate that *Doryctobracon areolatus* is more abundant then either *Diachasmimorpha longicaudata* or *Utetes anastrephiae* in the northern portion of the Caribbean fruit fly's inland range. *D. longicaudata* is more abundant in the south. Coastal situations are more complex.

Plans: Using biogeographic data for guidance, field and laboratory studies will be performed to discover temperature, humidity and other environmental preferences of the three species.

ATTRACTION OF *ANASTREPHA SUSPENSA* (DIPTERA: TEPHRITIDAE) TO VOLATILES FROM AVIAN FECAL MATERIAL

N.D. Epsky, B.D. Dueben R.R. Heath, and R.J. Prokopy[1]

Objectives: Laboratory trials were conducted to characterize the attraction of the Caribbean fruit fly to volatiles of avian fecal material and to determine the role of ammonia in this attraction.

Methods: Avian fecal material was obtained as droppings from housed chickens. Droppings were collected within 24 h of deposition and placed in storage at 4°C. Fecal material was removed from storage and held at room temperature and ambient relative humidity for 24 h before use. Fecal material was tested as crude material or as methanol extracts. Test substrates were added to 100 ml water for use in the bioassays. Factors of longevity of attractiveness (0-3 d), and physiological condition of females (virgin and mated) were investigated in flight tunnel bioassays. Subsequent bioassays investigated female response to material partially purified by solvent extraction. Finally, the role of ammonia in attraction to the fecal material was investigated by measuring release rate of ammonia and correlating female preference with ammonia release.

Results: Both virgin and mated females were trapped in response to volatiles from avian fecal material. In no-choice tests, response of mated females was higher on the first day (0 d) and last day of testing (3 d) than during the middle two days. In contrast, response of unmated females to crude material and response of both mated and unmated females to methanol extract remained fairly constant over all four days of the test. In choice tests, significantly more females, both unmated and mated, responded to volatiles from the crude material than from the extract for all but the 2 d old test solutions. There was a correlation between ammonia release rate and number of flies trapped in response to 0 d and 1 d old test solutions, but not in older solutions. Ammonia release from the 0 d crude fecal material solution was very high, but dropped almost four-fold in amount overnight and remained at that lower level throughout the remainder of the time period. Ammonia release rate from methanol extracts was significantly lower than release from the crude avian fecal material for the first three days of testing. Thus, ammonia release appeared to be the primary attractant on the first day of tests. Additional components, however, may add to this attraction, especially chemicals that are not released until the material has been aged for several days.

Plans: Bioassays will be conducted to further define the role of ammonia versus other volatile compounds that are emitted from avian fecal material. Candidate materials will be tested on both the caribfly and the apple maggot.

[1]Dept. of Entomol., Univ. of Mass.
Research was supported in part through a cooperative agreement with the University of Massachusetts at Amherst number 58-6615-3-018.

FIELD EVALUATION OF THE DRY TRAP WITH FOOD-BASED ATTRACTANT FOR THE MEDITERRANEAN FRUIT FLY

N.D. Epsky, R.R. Heath, W.L. Meyer[1], F. Geronimo[1], and C. Liera[1]

Objectives: Studies were initiated in Guatemala to evaluate the efficacy of the dry trap baited with synthetic food-based attractant, designated the Phase I dry trap, under various environmental conditions and to compare information obtained from medfly capture in the dry trap with information from the standard Jackson trap.

Methods: In February 1994, Jackson traps baited with trimedlure and dry traps baited with the medium dosage of ammonium acetate and putrescine were placed in two coffee fincas near Colomba, Guatemala. These are areas that are not currently under SIT release and are at an elevation of ~1500m. Coffee is the primary medfly host at both sites, and coffee fruit was abundant at the start of the study. Five lines of traps were placed at each site, with 50 m between each line. Five Jackson traps and 5 dry traps were placed in each line, with traps placed alternately 30 m apart along a line. Trap placement followed standard protocol and the experiment was run for 17 weeks. Trimedlure plugs were replaced every two weeks and the synthetic food-based lures used in the dry trap were replaced only if lost. Traps were checked weekly, and numbers of female and male medflies were recorded. All medflies trapped in the dry traps in a line were pooled and were placed in 70% isopropanol. These were shipped to Gainesville, where a subsample of females collected was dissected and mated status determined.

Results: Initially the Jackson traps captured more flies (males) than the dry trap, which captured both males and females, as was expected. However, approximately 50% of the medflies trapped in the dry traps were female, while only males were captured in the Jackson traps. During the last part of the study, the dry traps in one finca captured ~ 2 times as many flies as the Jackson traps. Jackson trap data suggested that the population was declining and the dry trap data indicated that the population of flies was not declining. On average, 20 and 25% of the females captured at each finca were mated. These results indicated that most of the females captured throughout the study were unmated.

Plans: The experiment has been expanded to examine trap performance at coffee fincas in two lower elevations and in sites with sterile medfly release. Data on temperature, fruit availability and larval infestation rates are also being collected. Improved traps will be incorporated into this study as they become available from our research.

[1]USDA APHIS Guatemala

[2]Partial support for this research is provided by USDA/OICD and a grant from the California Department of Food and Agriculture #91-0621.

PILOT TESTING USE OF GIBBERELLIC ACID FOR FRUIT FLY CONTROL IN CITRUS IN FLORIDA AND MEXICO

P. Greany, R. McDonald[1], and M. Aluja[2]

Objective: To use gibberellic acid to retard citrus fruit senescence and thereby delay the onset of fruit fly susceptibility.

Methods: Gibberellic acid (GA) treatments were applied to citrus trees in commercial groves using locally-appropriate spray equipment (speed sprayers in Florida, mechanically-powered hand sprayers in Mexico). Application was made prior to fruit colorbreak (in August in Florida, earlier in Mexico). Treatments were applied using 10-20 g of GA per acre, in combination with the surfactant Silwet L-77 at 0.05% (OSi, Inc.). Periodic samples of treated vs. untreated fruit are being made to assess leaf drop, fruit peel color and peel firmness. Bioassays of fruit susceptibility to fruit flies are being performed by exposing treated and untreated fruit together in field cages inoculated with Anastrepha suspensa (in Florida) or A. ludens (in Mexico). In addition, infestation by wild flies is being determined at each location by holding fruit from treated vs. untreated trees for fly development. Tests in Florida involve only 'Marsh' var. white grapefruit; in Mexico, tests are being conducted with both grapefruit and oranges.

Results: Application of 10-20 g/acre of GA in 250 gallons of spray solution had a demonstrable, dose-dependent effect upon initial leaf drop and upon fruit color and resistance to puncture both in Florida and in Mexico. Susceptibility of treated vs. untreated fruit to fruit fly attack is still being assessed in Florida. Results from Mexico suggest that GA treatment alone does not provide assured protection of grapefruit from the Mexican fruit fly, but along with use of malathion bait sprays, sufficient protection may be obtained. Mexican oranges rarely are attacked by *A. ludens*, except very late in the season. GA treatments reduced spontaneous abscission of Florida and Mexican grapefruit and oranges, enabling extended on-tree storage of the fruit.

Plans: To utilize the results obtained from these Pilot Test studies to allow citrus producers to improve their management capabilities for *Anastrepha* spp. at each location: (1) as a component of the fly-free protocol in Florida, and (2) for direct intervention against the *A. ludens* in Mexico, in combination with use of malathion bait sprays.

[1]U.S. Horticultural Research Laboratory, Orlando, FL
[2]Instituto de Ecologia, A.C., Xalapa, Veracruz, Mexico

HOBO TRANSPOSABLE ELEMENT GENE VECTOR ANALYSIS IN TEPHRITID FRUIT FLIES

A. M. Handler and S. P. Gomez

Objective: To test the mobility properties of the *hobo* transposable element in tephritid insects and to assess interactions with resident *hobo*-related systems in order to develop vectors and determine conditions necessary for gene-transfer.

Methods: *hobo* mobility was tested using a transient *in vivo* total excision assay in several tephritid fruit fly species and strains including *Anastrepha suspensa, Bactrocera dorsalis, Bactrocera cucurbitae, Ceratitis capitata, and Toxotrypana curvicauda.* Mobility was tested by a total excision assay involving the injection of an indicator plasmid, either alone or with a transposase-containing helper plasmid, into preblastoderm embryos. After incubation the plasmids were recovered and the ability of *hobo* element, carrying a reporter gene, to excise from the indicator plasmid was assayed. The indicator plasmid consisted of a lacZα peptide reporter gene inserted into the *hobo* open-reading frame in pHFL1. The helper plasmid was pHSH2, which has the *hobo* transposase open reading frame under hsp70 promoter regulation. Plasmids recovered from surviving embryos were transformed into bacteria by electroporation, which were plated on X-gal, kanamycin media allowing only indicator plasmid-transformed bacteria to survive. A lack of *hobo* excision retains the lacZα gene yielding blue bacterial colonies, while both precise and imprecise *hobo* excisions result in the loss of lacZα, and therefore β-galactosidase activity, yielding white colonies. Excision frequencies were computed by reaffirming excision events by restriction digest analysis and dividing the number of excision events by the total number of colonies. Some excision products were further analyzed by sequence analysis.

Results: In a previous study in *Drosophila melanogaster, hobo* excision was found to be completely dependent upon *hobo* transposase provided either by co-injection or by *hobo* within the genome of host embryos. In all the tephritid species tested *hobo* excision was detected at significant levels, though at varying frequencies, with or without the co-injection of helper. Of the twelve strains tested, only in the *C. capitata dark pupae* and *B. dorsalis white eye* strain was excision almost completely dependent upon co-injected *hobo* helper. Interestingly, in five of the strains tested, excision frequencies were significantly lower in the presence of co-injected *hobo* transposase. This indicates that 1) *hobo* mobility is permissive among tephritid insects, 2) *hobo*-like or other cross-mobilizable systems exist in these insects, and 3) *hobo* and the endogenous cross-mobilizing system may interact in a fashion that either inhibits *hobo* mobility or negatively affects DNA integrity proximal to *hobo* sequences. It is concluded that while *hobo* may function as a gene-vector in tephritids as well as other insects, cross-mobilizable systems might cause instability of *hobo*-mediated integrations or decrease host strain viability. In this respect optimal host strains would include those which lack *hobo*-related elements or have a low cross-mobilizing ability.

Plans: To test *hobo* gene-transfer vectors in *A. suspensa*, and the *C. capitata dark pupae* and *B. dorsalis white eye* strains.

STIMULATION OF *P* TRANSPOSABLE ELEMENT MOBILITY BY Γ-IRRADIATION

A.M. Handler, S.P. Gomez, and R.A. Hochstrasser*

Objective: To test the ability of γ-irradiation to stimulate *P* element excision in *Drosophila melanogaster* embryos in an effort to discover factors capable of promoting the activity of transposon-based gene transfer vectors in non-drosophilid insects.

Methods: *P* element mobility was tested in the *D. melanogaster* Engels 1 strain embryos, containing a single genomic copy of the somatically active Δ2-3 *P* element, after treatment with various doses of γ-irradiation. Excision was tested using a transient *in vivo* assay which measures precise *P* element excision from plasmids injected into host embryos. Pre-blastoderm embryos were collected and subjected to 0, 100, 200 or 400 rads γ-irradiation from a cesium source and were then immediately injected with the pISP2 excision indicator plasmid. M strain embryos (not containing *P* elements) were either non-irradiated or irradiated with 200 rads previous to pISP2 injection to control for non-specific (or non-*P* mediated) induction of excision. Excision was not detected in the M strain embryos, while excision in the Engels strain without irradiation occurred at a frequency of 0.3 x 10^{-3} excisions/ pISP2 indicator plasmids (consistent with previous assays). After irradiation excision increased two-fold at 200 rads and four-fold at 400 rads. Consistent with previous genetic data, a stimulatory or synergistic effect exists between γ-irradiation and *P*-element mobility. This suggests that irradiation may be used to promote activity of transposon-based gene vectors and possible stimulate more general transposon movement, facilitating the isolation of new transposable elements.

Plans: To test the influence in γ-irradiation on the mobility of the *P*-element and other transposons in nondrosophilid insects and on transformation in *D. melanogaster*.

*Olympic Heights Community High School, Palm Beach, FL
supported by Teacher Research Update Experience program

DEVELOPMENT OF A "DRY" PLASTIC INSECT TRAP WITH FOOD-BASED SYNTHETIC ATTRACTANT FOR THE MEDITERRANEAN AND MEXICAN FRUIT FLIES

R.R. Heath, N.D. Epsky, A. Guzman[1], B.D. Dueben, A. Manukian and W.L. Meyer[1]

Objectives: A new "dry" plastic insect trap was developed for use in monitoring populations of tephritid fruit flies. The plastic trap is constructed of acetate film that is painted to provide a visual cue. It contains a toxicant panel that provides a feeding stimulant to kill the flies after they have entered the trap.

Methods: The trap baited with a two component blend of ammonium acetate and putrescine was tested against feral populations of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and the Mexican fruit fly, *Anastrepha ludens* (Loew), in field trials conducted in Guatemala. Tests were conducted to determine 1) if both components were needed for optimal attraction and 2) if yellow, green, orange or clear (colorless) traps were optimal. Tests were then conducted to compare capture in orange and green plastic traps with capture in standard protein-baited McPhail traps. Separate comparisons were conducted with the plastic traps baited with low, medium or high dosage of synthetic bait. Numbers of fruit flies captured and mated status of a subset of females captured were determined.

Results: The combination of ammonium acetate and putrescine was better than either ammonium acetate-only, putrescine-only or unbaited traps. More female Mediterranean fruit flies were trapped in green traps than in clear traps, and capture of male Mediterranean fruit flies was greater in yellow traps than in orange traps. Neither female nor male Mexican fruit flies differentiated among orange, green and yellow traps, but percentage trapped in any colored trap was higher than in colorless traps. McPhail traps with standard protein bait caught more Mexican fruit flies than either of the plastic traps at any of the dosages of synthetics tested. However, plastic traps baited with either the medium or high dosage of synthetic blend caught equal numbers of Mediterranean fruit flies as McPhail traps. Although McPhail traps caught more female Mediterranean fruit flies than plastic traps baited with the low dosage of synthetic blend, more of the females captured in plastic traps were unmated (55-65% versus 22% in McPhail traps). Increase in dosage of the synthetic lure increased the percentage of mated females captured in the plastic traps. Unlike McPhail traps, which catch large numbers of miscellaneous Diptera, the plastic traps were highly specific and caught few non-target flies.

Plans: A manuscript describing this research has been accepted for publication in the Journal of Economic Entomology. Future research is directed towards improvement of this trap through changes to visual cue and chemical cue.

[1]USDA APHIS Guatemala

[2]Partial support for this research is provided by California Department of Food and Agriculture Grant #91-0621.

ERADICATION OF THE MEDITERRANEAN FRUIT FLY THROUGH COMBINED RELEASES OF PARASITES AND STERILE MALES

J.M. Sivinski and T. Holler

Objective: To test if Mediterranean fruit flies under Central American conditions can be more effectively eradicated by the addition of parasites to sterile fly releases.

Methods: Mediterranean fruit flies occurring in Latin America are a constant threat to billions of dollars of fruit and vegetable crops in the United States. The fly has been eradicated from Mexico, our nearest neighbor to the south, but Mexico is concerned with reinfestations from Central American countries. In order to form a fly-free buffer zone between Mexico and Guatemala and eventually eradicate the fly from the latter country, the USDA, the Guatemalan government and the international organization MOSCAMED, are collaborating in testing new means of Mediterranean fruit fly eradication. A mathematical model and an experiment in Hawaii suggest that this Central American effort might benefit from the combined releases of sterile male flies and fruit fly parasites. In order to test if this is an effective means of control under Guatemalan conditions, a large scale comparison of sterile fly and sterile fly and parasite releases is being made in coffee plantations in the mountains of southwestern Guatemala. Three parasitoid forms, *Diachasmimorpha tryoni*, large *D. longicaudata* reared on *Anastrepha ludens* and smaller *D. longicaudata* reared on *Ceratitis capitata*, are being individually released in 1 sq km plots at densities of 150,000/km^2. Sterile medflies are being released in these plots at densities of 300,000/km^2.

Results: Final results will not be available until mid-1995.

Plans: Releases over larger more isolated plots will begin in October 1995. Four other species of parasitoids will be colonized and their efficacy tested.

THE ECOLOGY AND BEHAVIOR OF NATURAL ENEMIES OF *ANASTREPHA* SPP. FRUIT FLIES

J.M. Sivinski and M. Aluja

Objective: To determine if certain natural enemies of *Anastrepha* spp. have specialized microhabitats and if the introduction of these specialized insects into the United States would improve the biological control of Caribbean fruit fly.

Methods: Research is being performed simultaneously in Florida and Southern Mexico. In each area, fruits from a variety of host trees are sampled from precisely determined locations, weighed and then held separately for insect emergence. In Mexico computerized sensors determine the light, temperature and humidity of different microhabitats in host trees every five minutes and log the data into memory packs that are retrieved weekly. At the same time, parasites and predators that attack flies in fallen fruit are sampled in containers whose different mesh-sized coverings exclude different types of natural enemies. If there are "gaps" in the foraging behavior of natural enemies present in Florida, they might be filled by importing specialized biocontrol agents from more diverse Mexican fauna.

Results: Preliminary results from the first of two years of sampling suggest that physical factors of the habitat, height, depth within the canopy and fruit weight influence the distribution of certain parasitoid species. However, this influence lessens as parasitism (competition) increases. Some parasitoids are more effective in utilizing larvae in large fruit and species also differ in their ability to locate infested fruits. Six species of parasitoids have been collected in Southern Mexico and these are in colony. These include a new species of *Coptera* (Diapriidae), which may only parasitize tephritids. If so, this could be an important candidate for inundative releases.

Plans: 1994 was the second year of a projected three year study. The microhabitats of further hosts will be described. Exploration for new biocontrol agents will continue and experiments on imported species will determine their suitability for introduction.

THE EFFECT OF EXPERIENCE ON FORAGING BEHAVIOR BY PUPAL PARASITOIDS OF TEPHRITID FRUIT FLIES

J.M. Sivinski

Objective: To determine if the host range of pupal parasitoids of Diptera can be influenced by larval host or adult oviposition experience, and if so, could such modifications be used to target hosts in augmented parasitoid release programs.

Methods: Two pupal parasitoid species *Dirhinus himalayensis* (Chalcidae) and *Spalangia gemina* (Pteromalidae), are being reared on both Caribbean fruit fly and house flies. After adult oviposition experiences they are placed in a bioassay chamber and given a choice of approaching either Caribbean fruit fly or house fly pupae. Pupal parasitoids of Diptera typically have broad host ranges. Although they have been used successfully as biological control agents in augmentation programs, they represent a danger to nontarget organisms. A restriction of host range through prior oviposition experience and the formation of a "search image" might increase their usefulness.

Results: Preliminary results suggest that both species prefer house fly over Caribbean fruit fly pupae. But that preference is diluted in *D. himalayensis* reared on Caribbean fruit fly.

Plans: A third species of pupal parasite, *Coptera* sp., will be similarly tested. This recently discovered insect is believed to be specialized to attack tephritid pupae.

ISOLATION AND IDENTIFICATION OF JUVENILE HORMONES FROM *ANASTREPHA SUSPENSA*

P.E.A. Teal.

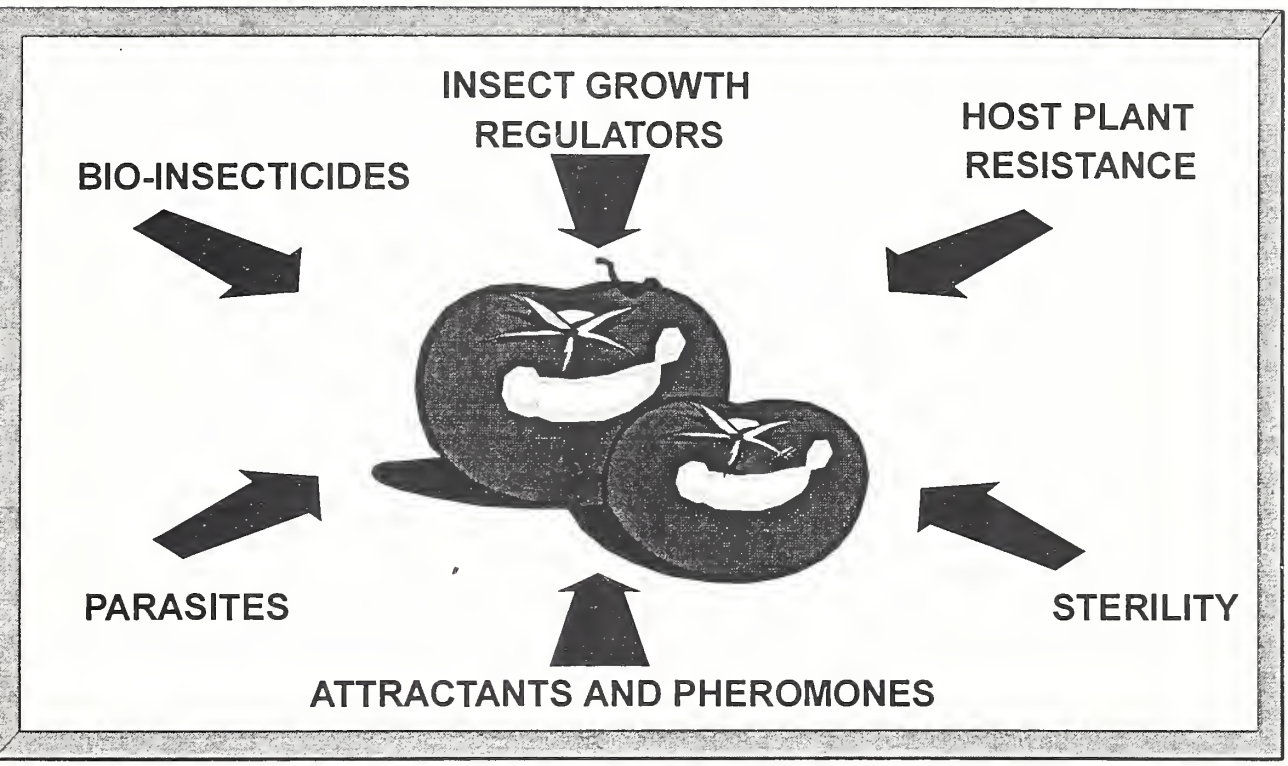
Objectives: To isolated and identify juvenile hormones from adult males and females of the Caribbean fruit fly, *Anaspreph suspensa*.

Methods: Adult males and females, segregated by sex on the day of emergence, were maintained in the laboratory for 10 days. During the photophase insects were collected and frozen on dry ice prior to storage at -60° C until 20 g had been collected. Flies were homogenized in acetonitrile and the homogenate was filtered. The acetonitrile filtrate was diluted by addition of an equal volume of H_2O containing 12% NaCl and extracted with an equal volume of pentane. The pentane extract was concentrated and subjected to solid phase extraction using a silica column. The column was washed with 30 ml of pentane followed by 30 ml of pentane containing 20% diethyl ether. Juvenile hormones (JH) were eluted using 15 ml of 50% diethyl ether in pentane. The fraction containing JH was concentrated and dissolved in acetonitrile prior to separation by HPLC. The initial HPLC separation was performed using a C18 reversed phase column with 50% acetonitrile in H_2O as the mobile phase. Fractions eluting at the retention volumes of synthetic JH I, II and III were collected and concentrated under nitrogen. Subsequent HPLC purification was conducted using a silica column. The fractions corresponding to JH I, II and III were dissolved in hexane and chromatographed individually using hexane containing 5% diethyl ether (H_2O saturated) as the mobile phase. Fractions corresponding the retention volume of the synthetic JH of interest were collected. The final HPLC separation was performed using a normal phase microbore column with hexane containing 0.6% acetonitrile as the eluting solvent. Purified samples were subjected to GC-mass spectroscopy using methane as a reagent gas for chemical ionization.

Results: To date we have completed the isolation, purification and identification of JH III from extracts of sexually mature males and females. JH III was identified from extracts of both males and females based on GC retention time and CI-MS fragmentation pattern. Approximately 0.4 pg of JH III was recovered from each male while ca. 1.0 pg was recovered per female.

Plans: Purified extracts of both sexes will be analyzed for the presence of other JHs and studies will be conducted to determine the effect of age on amounts of JH present in adult insects.

FIELD CROPS


INSECT GROWTH
REGULATORS
HOST PLANT
RESISTANCE
BIO-INSECTICIDES
PARASITES
STERILITY
ATTRACTANTS AND PHEROMONES

PHEROMONOTROPIC ACTIVITY OF A TOPICALLY APPLIED PYROKININ ANALOG IN *HELIOTHIS VIRESCENS*

R. Abernathy, P.E.A. Teal, and R.J. Nachman[1]

Objectives: To synthesize peptides with properties that allow them to cross the insect cuticle when topically applied and yet maintain biological activity.

Methods: An amphiphilic analog, [6-Pha-ala^0]Lom-MT-II, of the locust mytropic peptide Lom-MT-II, was synthesized. The amphiphilic nature of the peptide was imparted by attaching 6-phenylhexanoic acid to the amine terminus of the naturally occrring peptide. Standard bioassays for testing the pheromonotropic activity were conducted with 2-4 day old virgin females of *Heliothis virescens* by injection of 20μL of peptide dissolved in water. Doses ranged from 0.02-200 pmol per insect. Assays took place during the photophase when pheromone in not normally present. For topical assays, the scales were removed and 1 μL of the either pheromone biosynthesis activating neuropeptide, PBAN or [6-Pha-ala^0]Lom-MT-II solution was delivered onto the cuticle. Doses ranged from 5-1000 pmol per female. After the peptide was applied either topically or by injection, the insect was allowed to incubate at room temperature for 1 hour. The pheromone gland was then excised and analyzed by capillary gas chromatography for the major pheromone component of *H. virescens*, (Z)-11-hexadecenal (Z11-16:AL).

Results: The pheromonotropic activity of the amphiphilic peptide, [6-Pha-ala^0]Lom-MT-II, was first assessed by injection and compared to PBAN and Lom-MT-II. Lom-MT-II was very active at low doses. [6-Pha-ala^0]Lom-MT-II was less active than Lom-MT-II but comparable to PBAN. The peptides were dissolved in DMSO to evaluate pheromonotropic activity when applied to the cuticle. The activity of PBAN was less than that of [6-Pha-ala^0]Lom-MT-II. However, this showed that even larger peptides like PBAN can be topically applied in DMSO. Other solvents, 80/20% acetonitrile/water, 80/20% acetone/water and water, were compared for topical application of peptides. DMSO, 80% acetonitrile and 80% acetone flowed easily over the "de-scaled" cuticle. Water was difficult to apply as a blank or with PBAN dissolved in it. When [6-Pha-ala^0]Lom-MT-II was dissolved in water, however, the amphiphilic nature of the molecule reduced the surface tension of the water so that it wetted the cuticle much like the other solvents. The activity of [6-Pha-ala^0]Lom-MT-II in 80% acetone was too low for acetone to be considered for a solvent. [6-Pha-ala^0]Lom-MT-II in 80% acetontrile was active, but not as active as in water. Therefore, water was chosen as the solvent to be used in further assays. Topical application of PBAN showed appreciable activity only in DMSO, demonstrating that [6-Pha-ala^0]Lom-MT-II is crossing the cuticle independent of the solvent to stimulate pheromone production. A dose response for topical application of [6-Pha-ala^0]Lom-MT-II in water was linear. A timed study showed that [6-Pha-ala^0]Lom-MT-II began crossing the cuticle and stimulated the production of pheromone within 15 minutes. Between 1 and 2 hours, the activity began to decline.

Plans: The results of this study will be prepared for publication. Studies to elucidate the structural requirements of neuropeptides for stimulation of pheromone production are ongoing.

[1] USDA/ARS, College Station, TX.

IDENTIFICATION OF A FACTOR IN CATERPILLAR SALIVA THAT INDUCES CORN SEEDLINGS TO RELEASE VOLATILE PARASITOID ATTRACTANTS.

H.T. Alborn[1], J.H. Tumlinson, *and T.C.J. Turlings*[2]

Objectives: As a response to feeding by lepidoptera larvae, corn plants release volatile terpenoids, which attract parasitoids. This systemic release of volatiles is induced by a component that has been isolated from the foregut content of *Spodoptera exigua* larvae. Our present goal is to identify this component which has the potential of being a very useful tool in the study of parasitoid behavior and as a consequence also in the fields of plant resistance and plant breeding.

Methods: A purification protocol was developed that involves several steps of solid phase extraction and HPLC fractionation. A scaled up purification provided enough material for an attempt to identify the component. NMR and several soft ionization mass spectrometry - techniques, like FAB/MS, FAB/MS/MS and electrospray/MS were used to analyze the intact molecule. GC/MS and GC/FTIR were used to analyze derivatized fragments of the saliva factor.

Results: All the inducing activity could be related to one larvae produced component. The complex structure of the molecule made it necessary to use several complementary analytical techniques. An exact molecular weight measurement of the molecule could be used to establish a most likely elemental composition and the structure has been partially revealed.

Plans: The identification of the "saliva factor" will be completed, and a total synthesis of the molecule, or of biologically active parts of it will be considered.

[1]Department of Chemical Ecology University of Göteborg Sweden
[2]ETH Zürich Switzerland

SYNTHESIS OF BIOLOGICALLY ACTIVE CHEMICALS AND SEMIOCHEMICALS BY NEW EFFICIENT REGIO AND STEREOSELECTIVE ROUTES

R.E. Doolittle, D. Patrick, and H. Alborn

Objectives: To selectively synthesize a series of five branched chain C35 hydrocarbons as candidates for host-locating contact kairomones of *Brachymeria intermedia*, a parasitoid of Gypsy Moth pupae. To establish the structure/activity relationship that determines the chemical basis of biological activity of these types of molecules.

Methods: The synthesis route employed for the second analogous pair of branched chain hydrocarbons (the five hydrocarbon candidates consist of two analogous pairs and one candidate, differing from one another in the branching positions) is distinctly different from that used for the preparation of the first analogous pair (Sonnet, P.E., J. Am. Oil Chem. Soc., 53(2):5759, 1976) and several closely related compounds. The synthesis used the procedures published by Pomonis, J. G., et al., J. Chem. Ecol., 15(9):2319-2333, 1989. This consists of the acylation of thiophene with a long chain acid chloride in the presence of tin IV chloride. The resultant ketone is converted to an olefinic substituted thiophene which is reduced to an alkyl substituted thiophene, which is again acylated with a different long chain acid chloride. This ketone is alkylated adjacent to the carbonyl and subsequently subjected to a modified Wolff-Kishner reduction to remove the oxygen, raney nickel reduction for sulfur removal, and finally reduction of the resultant dienes to the saturated target molecules. Since each candidate molecule consists of a mixture of four diastereomeric isomers, and since none of the original five candidates, which could have been present in the isolated mixture, gave bioassay activity equivalent to the crude or purified natural product, we undertook a partial fractionation of the two most active compounds prepared thus far. This consisted of repeated fractional crystallization of these two mixtures from hexane.

Results: In the original published alkylation of the ketones, very poor yields were obtained. We have dramatically improved the yields of this step, by the use of cosolvents and improved techniques. Using these improved modified procedures, 7,13-dimethyltritriacontane, 9,15-dimethyltritriacontane and 7,15-dimethyltritriacontane were prepared in high purity (98% by GC) and good overall yield. Sufficient quantities of several strategic intermediates were prepared (as was done in the synthesis of the first pair of hydrocarbons) so that analogs can be efficiently prepared when desired. When the first pair of analogs was prepared, four additional structurally related compounds were synthesized and bioassayed. One of these, 11,15-dimethylpentatriacontane, showed greater activity in the biological evaluations than the isolated materials. We have synthesized a total of seventeen dimethyl branched hydrocarbons utilizing both synthetic approaches (see methods) with sufficient structural variation to provide a meaningful structure/activity relationship when bioassay data is complete. Repeated fractional crystallization of two of the synthetic hydrocarbons produced four diastersomerically biased mixtures for bioassay. These are being bioassayed presently.

Plans: There are no plans to synthesize additional molecules with structured variations, but pending the bioassay results from the diastereomerically biased mixtures that were prepared the synthesis of one or more hydrocarbon structures in diastersomerically pure form is contemplated and is being planned.

SYNTHESIS OF POTENTIAL INHIBITORS (SYNERGISTS) OF PHEROMONAL ATTRACTION IN THE CABBAGE LOOPER, *TRICHOPLUSIA NI* (HÜBNER) AND THE CORN EARWORM, *HELICOVERPA ZEA* (BODDIE)

R.E. Doolittle and M. Mayer

Objectives: To synthesize branched chain analogs of the pheromones of these two pest insects and electrophysiologically evaluate these compounds. It has been shown (Buager, B.V., et al., Tett. Lett., 40:5771-5772, 1990) that a branched chain isomer of the pheromone of the false codling moth, *Cryptophlebio leucotreta,* strongly inhibits the response of this insect to its synthetic pheromone. The pheromone of this insect is a blend of (E)- and (Z)-8-dodecenyl acetate in a 1:1 ratio. The inhibitor is 7-vinyldecyl acetate. The cabbage looper and the corn earworm utilize similar types of pheromones, (Z)-7-dodecenyl acetate and (Z)-11-hexadecenal respectively. A behavioral and neurophysiological examination of the effects of compounds analogous to the inhibitor found for the false codling moth was undertaken.

Methods: The syntheses of several branched chain analogs was undertaken. This consisted of alkylation of an aliphatic acid with alkyl halides containing masked alcohols, followed by reduction of the acids to alcohols and oxidation to an aldehyde. The aldehydes are extended to a terminal olefin and the alcohols unmasked and acetylated to produce the target molecules. The acetates in turn can be used to prepare other types of functional groups such as alcohols and aldehydes (specifically the branched analogs of (Z)-11-hexadecenal. The synthesis is designed to allow the preparation of the final products in optically pure form if so desired. The responses of antennal sex pheromone specialist receptor neurons in Cabbage Looper, *Trichoplusia ni* (Hübner) to these chemicals alone and in combination with pheromone (Z)-7-dodecenyl acetate) were recorded with tungsten electrodes that were inserted at the base of sensilla that house the neurons. The five target molecules that were synthesized and purified were subjected to extensive neurophysiological examination and to extensive behavioral evaluation in wind tunnel bioassays. The same general synthetic methods were employed for the preparation of additional quantities of the two initial target molecules for behavioral field tests. Thesesame synthetic methods were used to prepare structural variations of the original target molecules for molecular structure versus activity efforts.

Results: The response of the electrophysiological and behavioral experiments that established the presence of synergism in the response of the HS (a) receptor specialist neuron of *Trichoplusia ni* was put into manuscript form and submitted for publication in Science.

Since the response of the specialist receptor neuron of *Helicoverpa zea* also was strongly synergized by the two pheromone mimics that were initially prepared for the *T. ni* work, additional chemicals were synthesized for evaluation with both receptor neurons. Six additional structural variations of the original two synergistic target molecules were synthesized and purified for evaluation of synergistic effectiveness with the receptor neuron of *H. zea.* In addition, the synthesis of eight additional structural variations of the original target molecules was undertaken and each is partially completed.

Plans: Additional quantities of the synergistic molecules will be prepared for the running of additional tests including field tests with the two target insects. In addition, neurophysiological and behavioral evaluation of these chemicals will be conducted with other insect pests and/or with other collaborators with the goal being investigation of the scope of this effect. Other candidate molecules with structural variations of the original target molecules will be synthesized and evaluated against these two pest species and others to measure the effect of a molecular structural variation on the synergistic effect.

INVESTIGATION OF THE REPLACEMENT OF THE HIGHLY CARCINOGENIC SOLVENT, HEXAMETHYLPHOSPHORAMIDE IN PHEROMONE SYNTHESIS

R.E. Doolittle and C. Castro

Objective: To determine whether the highly carcinogenic solvent, hexamethylphosphoramide (HMPA) can be replaced by less toxic solvents in pheromone and related syntheses. HMPA has long been used as a solvent in many of the alkylation reactions that are an integral part of the synthesis of pheromones and other biologically active molecules. However, clinical evidence strongly indicates that HMPA is a potent carcinogen, prompting intensive investigations aimed at replacing it with less toxic solvents. Two solvents that have been reported to serve as good replacements for HMPA are 1,3-Dimethyl-2-imidazolidinone (DMI) and 1,3-Dimethyl-3-4,5,6-tetrahydro2(1H)pyrimidinone (DMPU). These two solvents also have been evaluated in clinical trials and are much less carcinogenic than HMPA.

Methods: Nonanoic acid was α-alkaylated with 1-bromobutane using HMPA, DMPU and DM1 in the molar amounts reported, and under identical conditions reported in the original publication from 1972 which used only HMPA. We found that using the same molar equivalent of HMPA, DMPU and DMI produced good yields of the α-alkylated acid (80-90%) but the DMPU and DMI gave yields about 10% less than the HMPA. Using two molar equivalents of DMI increased the yield from 80 to 85%. The results obtained thus far indicated that DMI and DMPU are good replacements for HMPA in the α-alkylations of aliphatic acids but it may be necessary to use molar equivalents larger than those used with HMPA to obtain equivalent yields.

In addition, the alkylation has been extended to other acids in order to test the general applicability of the two replacement solvents.

Plans: The use of DMI and DMPU in the alkylation of aliphatic acids will be completed and the use of these two solvents in preparation of branched phosphonium salts will be investigated. Practical aspects of replacing HMPA with DMI and DMPU and other types of alkylation reactions that are routinely used in pheromone synthesis will be evaluated.

ECONOMICAL REARING OF *MICROPLITIS CROCEIPES* ON ATYPICAL HOSTS: EVALUATION OF METHODS TO INHIBIT PARASITOID ENCAPSULATION AND IMPROVE ADULT EMERGENCE

S.M. Ferkovich, P. Gupta and C.R. Dillard

Objective: To test the effects of host exposure to radiation (cesium source), extreme rearing temperatures, and azadirachtin on encapsulation and emergence of *M. croceipes*.

Methods: An obstacle to rearing parasitoids on atypical hosts is the host's immune response or encapsulation of the parasitoid egg and larvae by the host's hemocytes. Potential methods of inhibiting this response were examined in three atypical hosts. The typical host, *Helicoverpa zea*, along with three atypical hosts *Spodoptera frugiperda, Spodoptera exigua*, and *Galleria mellonella* were exposed to temperature extremes (30° C to 42° C), gamma radiation (20 to 100 greys) for 24 hours prior to exposure to parasitoid females. The same species were also maintained on diets supplemented with azadirachtin (0.1 to 10 ppm of the growth inhibitor) in anticipation of suppressing the host's immune system.

Results: None of the treatments of the atypical hosts with extreme temperatures, gamma radiation, and azadirachtin enhanced parasitoid emergence. The radiation treatments significantly reduced adult emergence at 20 grays and higher doses. Azadirachtin did not significantly increase parasitoid emergence over controls and was not significantly detrimental to the parasitoid although it caused higher host mortality.

Plans: A manuscript is in preparation. The effect of injection of a polydnavirus associated with the wasp egg on suppressing the immune response and on growth and development of the natural host, *H. zea*, and also in *Galleria* will be determined. Also, we will examine and compare the level of polydnaviral DNA expression in *H. zea* with that in *Galleria* and *Spodoptera exigua*.

ECONOMICAL REARING OF *MICROPLITIS CROCEIPES* ON ATYPICAL HOSTS: POTENTIAL OF AN UNNATURAL HOST, *GALLERIA MELLONELLA*.

S.M. Ferkovich, P. Gupta and C.R. Dillard

Objective: To improve parasitism of *Galleria mellonella* by *M. croceipes* and to compare development of the parasitoid with its' development in the natural host, *H. zea*.

Methods: *Microplitis* females were stimulated to oviposit in atypical hosts by treating them with typical host hemolymph and frass. Freeze-dried hemolymph and the host seeking stimulant, 13-methylhentriacontane, were tested in combination to enhance oviposition in G. mellonella. The stung hosts are then held to observe development of the parasitoid and for adult parasitoid emergence. The host diet was supplemented with the following nutritional supplements to enhance development of the parasitoid: torula yeast, liver, fetal bovine serum, chicken seum, freezed-dried *H. zea* hemolymph, powdered Grace's and TC-100 cell culture media.

Results: Parasitoid females oviposited in early fifth instar *G. mellonella* larvae after treating them with hemolymph and frass from *H. zea*; however, the rate of parasitism of *G. mellonella* was 41%, compared with 90% on third instar *H. zea* larvae. Percent parasitization of *Galleria* was significantly improved from 41% to 90% which is comparable to that in *H. zea* by using freeze-dried hemolymph and purified host-locating kairomone, 13-methylhentriacontane (Fig. 1). The sex ratio of parasitoids reared on *G. mellonella* (40% male: 60% female) was not significantly different from parasitoids reared on *H. zea* (35% male: 65% female). Parasitoid cocoons weighed 8.30 $\pm$ 0.34 mg SE/cocoon compared with 14.08 $\pm$ 0.16 mg SE/cocoon from *H. zea*. Based on measurements of metathoracic tibia, femur and forewing length, F_1 adults reared on *G. mellonella* were smaller than those reared on *H. zea*. Although the size differences were thought to be due to suboptimal nutrition, supplementing the diet of *Galleria* slightly improved parasitoid emergence. When the F_1 progeny of *G. mellonella*-parents were reared back on the natural host, *H. zea*, the parasitoids returned to normal size.

Plans: A manuscript has been submitted to journal. The effect of injection of a polydnavirus associated with the wasp egg on suppressing the immune response and on growth and development of the natural host, *H. zea*, and also in *Galleria* will be determined. Also, we will examine and compare the level of polydnaviral DNA expression in *H. zea*, with that in *Galleria*, and *Spodoptera exigua*.

ARRESTMENT RESPONSE OF *TELENOMUS REMUS* (SCELIONIDAE: HYMENOPTERA) BY A KAIROMONE ASSOCIATED WITH ITS HOST EGGS

Y. Gazit, W. Joe Lewis, and J. H. Tumlinson

Objectives: To characterize the arrestment response of the parasitoid caused by a kairomone found on its host eggs. To isolate this kairomone and to identify its chemical structure.

Methods: *Telenomus remus* were reared on eggs of the fall army worm, *Spodoptera frugiperda*. A bioassay was modified to monitor the arrestment response of the wasps to hexane extracts of the kairomone. The retention of the wasp on a 1 cm^2 filter paper, treated with the kairomone, was timed with a stopwatch. experiments were performed using 2- day old, naive wasps. This bioassay was employed to characterize the arrestment response of the wasp. Liquid chromatography techniques, such as silica gel column and normal phase HPLC, were used to partially purify the active compound. Active fractions in the purification procedure were detected using the bioassay.

Results: A contact kairomone that caused an arrestment response by the egg parasitoid, was found associated with its host eggs. The kairomone was very active and a significant response was induced with an extract equivalent to a single egg. Only the female wasps reacted and in a dose dependent manner. Arrestment activity was also found in hexane extracts of the whole body of adult moths, and of the abdominal tip, which contains the pheromone gland. The active compound in the egg extract was partially purified. It was found to be a non polar substance, probably not a saturated hydrocarbon.

Plans: The biological activity of the arrestment kairomone found on the eggs will be further characterized using the bioassay. Several chromatography techniques will be used to further isolate and purify the active compounds. Their chemical structures will be elucidated by spectroscopic techniques. We anticipate that the accumulated knowledge about the characteristics of the arrestment response, as well as the chemical nature of the kairomone, will be used for manipulation procedures of the egg parasitoids in modern biological pest control.

DEVELOPMENT OF ARTIFICIAL MEDIA AND REARING SYSTEMS FOR AN ECTOPARASITOID OF *SPODOPTERA SPP.* AND A PREDATOR OF THE GYPSY MOTH

P. Greany, J. Carpenter[1], R. Weseloh[2] and J. Venn

Objective: To develop low-cost artificial media and presentation systems to enable mass rearing of the *Spodoptera* pupal parasitoid *Diapetimorpha introita* (Hym.: Ichneumonidae) and the gypsy moth predator, *Calosoma sycophanta* (Col.: Carabidae).

Methods: Artificial media are being prepared by combining readily-available, low-cost materials of non-insect origin to simulate the biochemical composition of lepidopteran larvae or pupae. The media are being encapsulated in a form that allows for host/prey recognition and acceptance by the parasitoid and predator in question. Details of preparation and processing techniques will be provided in a future patent application.

Results: Approximately 60-70% of *D. introita* neonate larvae provided with the artificial host are able to complete development and form apparently normal male and female adults. The rate of development is almost comparable to those reared on *Spodoptera* pupae. Larvae of *C. sycophanta* beetles have grown to adults using the artificial medium, and these adults are producing viable eggs.

Plans: Bioassays will soon be conducted to evaluate the host-seeking propensity, host-finding success, fecundity, and longevity of artificially-reared *D. introita* and *C. sycophanta* individuals. Tests will be conducted to determine whether the media will support continuous generations of these entomophages. A CRADA has been established with Predation, Inc., of Gainesville, FL, to commercialize the process for mass-rearing of these entomophages.

[1]USDA ARS Insect Biology & Population Management Research Laboratory, Tifton, GA
[2]Connecticut Agricultural Research Station, New Haven, CT

SUITABILITY OF PLANTS OF NORTH CENTRAL FLORIDA AS HOSTS FOR LARVAL SOYBEAN LOOPERS, *PSEUDOPLUSIA INCLUDENS*

P.J. Landolt and S. Lovvorn

Objective: To identify good host plant species for the soybean looper by comparing larval survival, development rates, and pupal weight for different plant species.

Methods: Plants were selected locally from a variety of habitats, or were grown on site in a garden from purchased seed or seedlings. Emphasis was placed on cultivated herbaceous crops and herbaceous weeds. Each plant was tested by placing cut foliage in five 8 oz wax coated paper cups with 5 newly hatched soybean larvae per cup (n=25). Soybean looper larvae used for most of these tests were obtained from eggs shipped from the USDA, ARS insect rearing facility in Stoneville, Mississippi. On each day thereafter, foliage was added or replaced as needed (if dried, dead, moldy). Data were recorded for larval numbers, pupation, and adult emergence. Pupae were sorted by sex and were weighed.

Results: To date, eighty-two species or varieties of plants have been evaluated, including 19 crop cultivars. Highest rates of survival from first instar larvae to adult were obtained for larvae reared on the weeds ironweed (80%),wild radish (72%)curly dock (88%), and marsh pennywort (88%), and on the crops cotton (96%), cowpea (76%) and lima bean (70%). Greatest pupal weights were obtained with larvae on chicory (239 mg), sow thistle (245 mg), tobacco 299 mg), and potato (265 mg). Fastest development time (to adult) was obtained for larvae reared on peppermint (21 d), cowpea (21 d), cabbage (21 d) and basil (22 d). Of the 82 plants tested, soybean loopers completed development on 54 (63%). As with other studies with the cabbage looper, a greater percentage of cultivated plants tested some supported complete development of soybean looper (89%), compared to non-cultivated plants (56%). In comparison to data on the cabbage looper, the soybean looper appears to be much more polyphagous.

Plans: Work is continuing to obtain the same data for more species of plants, to match similar studies for the cabbage looper. A direct comparison will be made of the performance of wild versus colony soybean loopers on intermediate host plants to determine if colony insects have altered development on wild host plants.

EFFECTS OF CABBAGE LOOPER LARVAL FEEDING DAMAGE ON CABBAGE LOOPER MOTH ATTRACTION TO, AND OVIPOSITION ON, COTTON FOLIAGE

P.J. Landolt

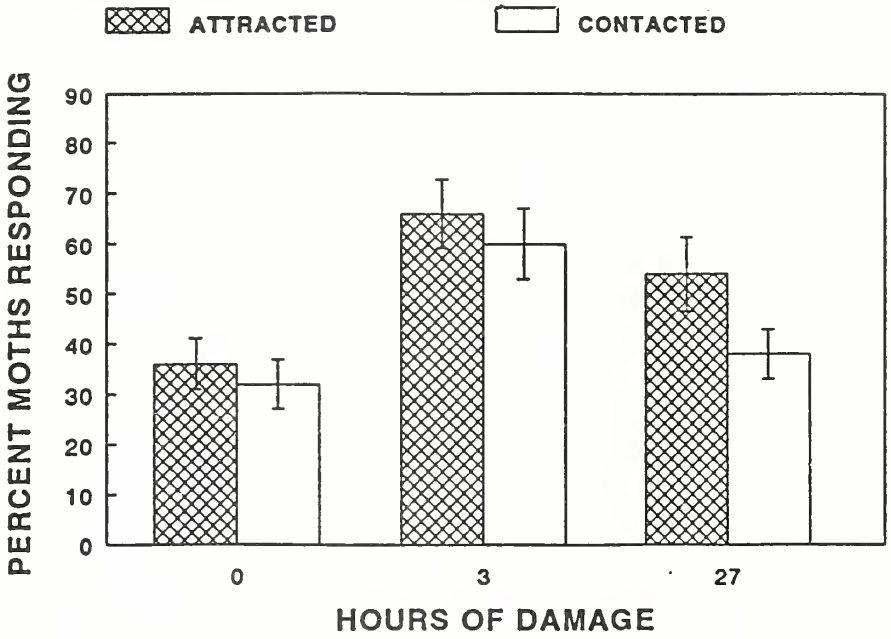
Objective: To determine if cabbage looper moths exhibit greater attraction to, and reduced oviposition on, foliage of cotton plants that were damaged by cabbage looper larvae, compared to undamaged cotton foliage. Previous tests of the effects of plant damage on moth attraction responses were done with moths on a reversed light cycle and plants on a natural light cycle. In this experiment moths and plants were synchronized under natural light regimes.

Methods: Cotton plants (Germaine 510) were grown from seed in a greenhouse and were tested as intact potted plants. Cabbage looper pupae and emerged moths were held in screened cages in another greenhouse room. Plants with no damage were compared with plants fed upon by 5 fourth instar cabbage looper larvae for 3 hours or for 27 hours preceding tests of attractiveness or for oviposition. Attraction responses of mated female cabbage looper moths to plants were evaluated in a flight tunnel, with all moths tested individually for a 2 minute period. Moths were scored for plume tracking responses and for contact with the plant. Series of 5 moths were tested to each of the three plant treatments on a given day, with the series repeated over 5 days, providing a total of 25 moths tested per treatment. Oviposition on plants with no damage, 3 hours of damage, or 27 hours of damage was compared in a large field cage, with a plant of each treatment category presented simultaneously on the cage floor. Ten mated female cabbage loopers were released into the cage at dusk and eggs on each plant were counted the following morning. This assay was repeated 6 times, with treatment positions in the cage rotated each day.

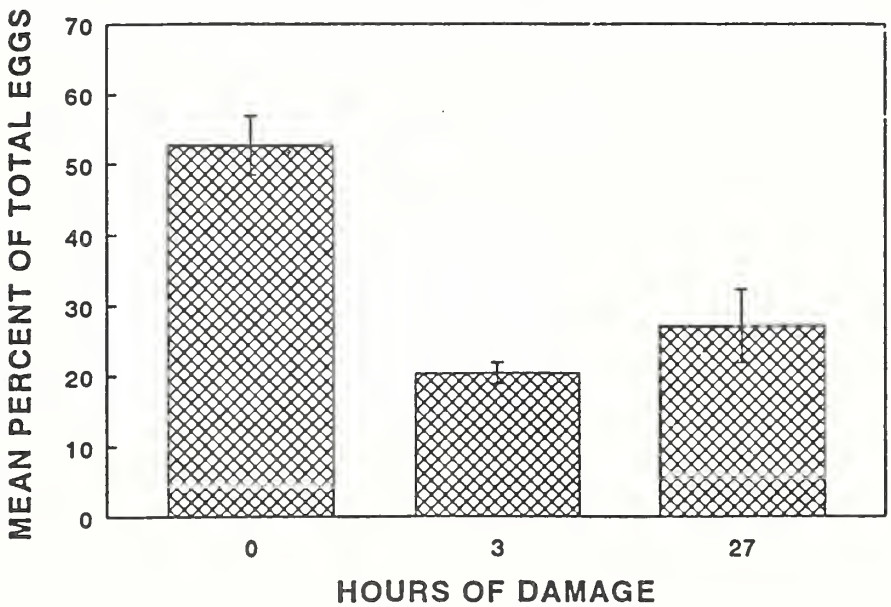
Results: Attraction of cabbage looper moths to cotton plants was greater when plants had been fed on by larvae for 3 hours, compared to undamaged plants or plants fed on for 27 hours (Figure 1). Oviposition was reduced on damaged plants compared to undamaged plants (Figure 1). These findings are similar to those reported previously for experiments conducted with moths and plants on different light cycles.

Plans: Cotton foliage odorants known to arise from short term damage to plants is being tested for attractiveness to mated female cabbage looper moths.

MATED FEMALE T. NI ATTRACTION TO LARVAL DAMAGED COTTON PLANTS



MATED FEMALE T. NI OVIPOSITION ON LARVAL DAMAGED COTTON PLANTS



EFFECTS OF FOOD DEPRIVATION ON ATTRACTION OF UNMATED FEMALE CABBAGE LOOPERS TO MALE PHEROMONE

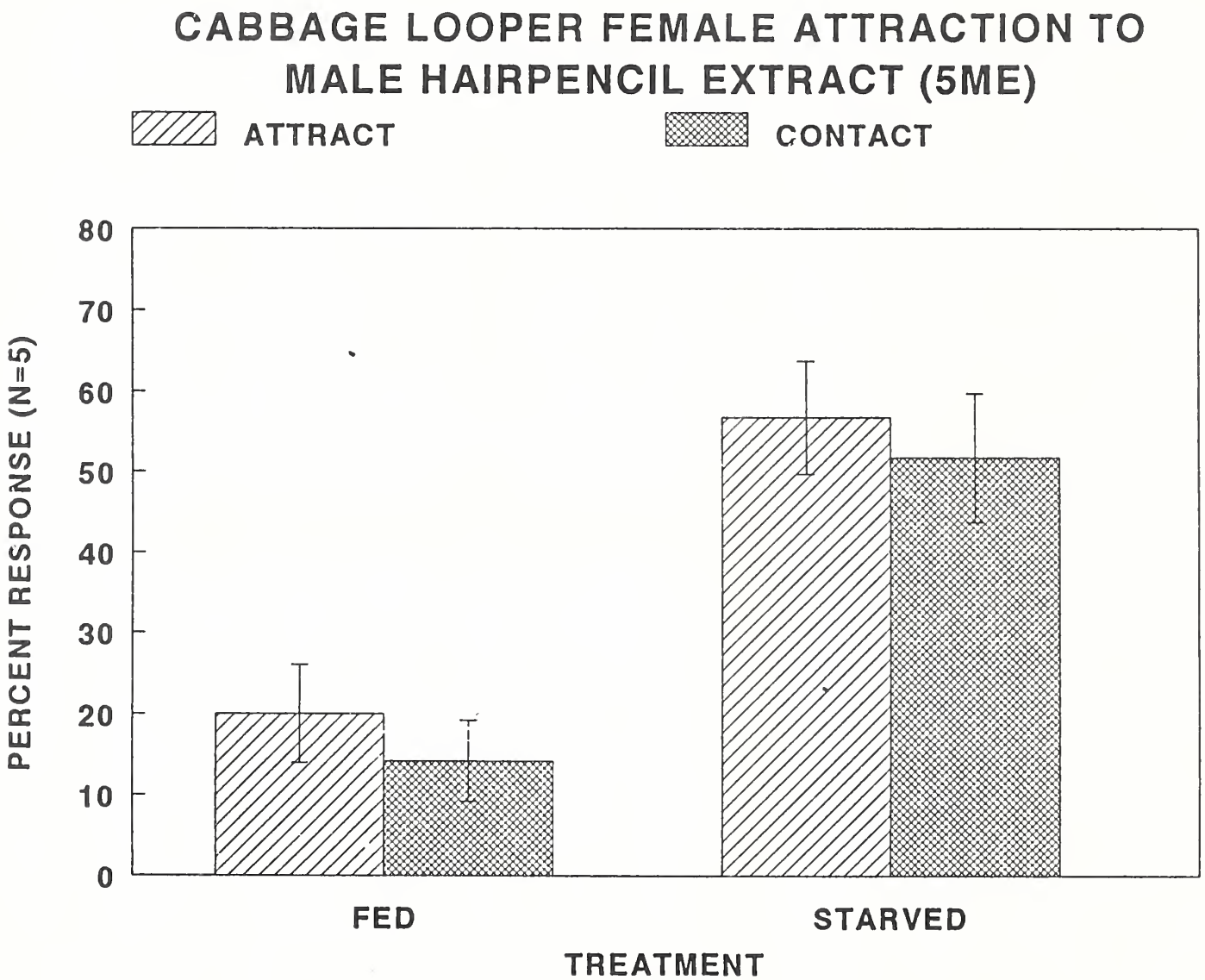
P.J. Landolt and O. Molina

Objective: To determine if starved female cabbage loopers are attracted more to male pheromone than fed females, as might be expected if females respond to males to obtain access to food.

Methods: Moths from the laboratory colony were used in this study. Male pheromone was obtained by removing the terminal hairpencils from 3 to 4 day old males and extract with hexane. Male pheromone was tested at a 5 male equivalent dose in 200 μl hexane applied to a filter paper. Batches of newly emerged females were separated into 2 groups, with one group given both water on cotton and a sugar-honey mixture on cotton as food, and the other group given only access to water. Female moths were tested for attraction responses in a flight tunnel, with a series of 5 females tested (for 2 minutes each) for responses to each application of male pheromone on a paper. Sets of fed females were compared with sets of starved females for rates of attraction responses. Moths tested were scored in the bioassay for plume tracking (upwind oriented flights within the odor plume) and for contact with the filter paper. Twelve comparisons of fed versus starved moths were made, for a total of 60 fed and 60 starved females tested for attraction to male pheromone. Mean response data calculated for the twelve sets were compared by Student's t-test for differences between treatments.

Results: Female cabbage loopers that were deprived of any source of food responded to male pheromone with attraction to contact the filter paper (51.7%) significantly more often than did female moths that were provided a mixture of sucrose and honey (14.2%). This data supports the hypothesis that females are attracted to male pheromone to obtain access to food.

Plans: Experiments will be conducted to evaluate the effects of food deprivation on male cabbage looper attraction to male pheromone, and the effects of food deprivation on the attractiveness and pheromone production of males.


CABBAGE LOOPER FEMALE ATTRACTION TO
MALE HAIRPENCIL EXTRACT (5ME)
ATTRACT
CONTACT
PERCENT RESPONSE (N=5)
80
70
60
50
40
30
20
10
0
FED
STARVED
TREATMENT

SUITABILITY OF PLANTS OF NORTH CENTRAL FLORIDA AS HOSTS FOR LARVAL CABBAGE LOOPERS, *TRICHOPLUSIA NI*

P.J. Landolt and S. Lovvorn

Objective: To identify good host plant species for the cabbage looper by comparing larval survival, development rates, and pupal weight for different plant species.

Methods: Plants were selected locally from a variety of habitats, or were grown on site in a garden from purchased seed or seedlings. Emphasis was placed on cultivated herbaceous crops and herbaceous weeds. Each plant was tested by placing cut foliage in five 8 oz wax coated paper cups with 5 newly hatched cabbage larvae per cup (n=25). On each day thereafter, foliage was added or replaced as needed (if dried, dead, moldy). Data were recorded for larval numbers, pupation, and adult emergence. Pupae were sorted by sex and were weighed.

Results: Two hundred and eight species or varieties of plants were evaluated, including 31 crop cultivars. Highest rates of survival from first instar larvae to adult were obtained for larvae reared on the weeds chicory (72%), common sow thistle (72%), false dandelion (80%), and ironweed (76%), and on the crops sweet potato (76%), collard (96%), cauliflower (76%), carrot (92%), and cowpea (70%). The highest pupal weights were obtained with spiny amaranth (207 mg), carrot (201 mg), chicory (224 mg), salsify (202 mg), wild radish (213 mg) and cotton (232 mg). Fastest development time (to adult) was obtained for larvae reared on wild radish (22 days), snow pea (22 d), chicory (23 d), marigold (23 d), salsify (23 d), lambsquarters (23 d), cabbage (23 d), and potato (23 d). Of the 208 plants tested, cabbage loopers completed development on 46 (22%), but fed on 162 different kinds of plants (78%). A greater percentage of cultivated plants tested supported some complete development of cabbage looper (61%), compared to non-cultivated plants (15%). For non-cultivated species of plants, the greatest number of good hosts were in the Asteraceae, particularly the lettuce subfamily (chicory, salsify, wild lettuce, sow thistle, false dandelion).

Plans: Work is continuing to obtain the same data for more species of plants, particularly cultivated vegetables and herbs. Additional studies planned include the sampling of wild host plants in the field to obtain host utilization data, and the testing of weed species that are good hosts for attraction and oviposition responses in the laboratory and for effects on sex pheromone behavior.

SEARCHING BEHAVIOR OF *MICROPLITIS CROCEIPES* FOR EXTRAFLORAL NECTAR (EFN) AS A FOOD SOURCE ON DIFFERENT COTTON VARIETIES

G. Makranczy, U.S.R. Röse, W.J. Lewis, and J.H. Tumlinson

Objectives: Preliminary wind-tunnel choice tests have shown that female *Microplitis croceipes* wasps preferred nectaried over nectariless cotton plants, and those that had been previously experienced with nectar, stayed much longer on these plants. The objective of our study was to further investigate the food foraging behavior of the parasitic wasp *M. croceipes* and its ability to learn to distinguish between nectaried and nectariless cotton varieties.

Methods: Potted nectariless (Stoneville 825) and nectaried (Delta Pineland 90) cotton varieties (*Gossypium hirsutum*), in the 4- to 6-leaf stage were used in the experiments. A plant was placed in a plexiglass cage (25cm x 30cm x 45cm) with a front hole and 3- to 6-day-old mated female wasps that were previously experienced on extrafloral nectar in petri-dishes or naive wasps were placed either on the upper or on the lower side of a leaf. All wasps were starved for approximately 24 hours prior to the experiment. The frequency and duration of resting, preening, walking, antennating and feeding behavior of the wasps was recorded using the OBSERVER software (Noldus Information Technology) and each insect was tested three times for 300 seconds each time.

Results: Nectar experienced wasps that were released onto the lower surface of nectaried cotton leaves were most successful at finding the nectaries (80%). Previously non-experienced, or wasps released on the upper side of the leaf, were equally unsuccessful (only 20-25% found the nectary). When placed on the upper side of the leaf the average time spent by experienced wasps on nectaried plants was 281s compared to only 126s spent on the nectariless varieties. Naive insects did not show this difference: 224s and 223s spent on the nectaried and nectariless plants, respectively. In the case of nectariless plants, wasps released on the lower surface of the leaf spent twice the time with active searching there, indicating that the wasps may not only learn the odor of the nectar, but also its location. Thus wasps that experienced nectar on the bottom of a petri-dish might expect to also find the nectary on the top surface of a leaf. In general, experienced insects were more successful at finding the nectar and also spent less time on nectariless than on nectaried cotton plants. The naive wasps spent more time on nectariless plants than experienced insects.

Plans: We plan to determine whether the wasps have the ability of visual mapping and if gravity could play a role in the searching behavior.

SYNERGISM OF THE Z11-16:AL SPECIALIST RECEPTOR NEURON OF *HELIOTHIS ZEA* (BODDIE) BY A SEX PHEROMONE COMPONENT AND SEVERAL SYNTHETIC PHEROMONE ANALOGUES BUT NOT BY HYDROCARBONS

M.S. Mayer and R.E. Doolittle

Objective: To determine whether or not a sex pheromone component, several synthetic sex pheromone analogues and hydrocarbons synergize the response of the neuron specialized to detect Z11-16:Al on the antenna of the corn earworm, *Heliothis zea* (Boddie).

Methods: Responses of (Z)-11-hexadecenal (Z11-12:Al) specialist receptor neurons of the corn earworm moth antenna were recorded extracellularly with a sharpened tungsten electrode. The action potentials were digitized, recorded and counted by means of a proprietary computer program. The stimuli chosen were assayed alone and as admixtures with Z11-16:Al.

Results: The response of the receptor specialist neuron was synergized by the sex pheromone component, Z7-16:Al, all of the sex pheromone analogues, but not by any of the hydrocarbons. As in the cabbage looper the amount of synergism was dependent upon the concentration of both Z11-16:Al and the synergen.

Conclusions: Based on current concepts synergism is the result of a simultaneous or near simultaneous multicomponent interaction. This newly discovered type of sex pheromone interaction suggests that the interaction may be either at one receptor site or at other site(s) on the same receptor macromolecule. It is significant that hydrocarbons did not elicit a synergized response because they lack particular physical and chemical parameters that appear to dominate these interactions. There may be an evolutionary basis for this interaction. A practical use for this knowledge is that a synergist may be used as an additive to compounds that are either expensive or labile for use in field disruption dispensers.

Plans: The electrophysiological responses of individual receptor neurons in other species will be assayed with similar synthetic analogues, sex pheromone components, and host plant compounds to determine the range of potentially synergistic compounds. Further structure-activity studies are planned to gain an insight into the pharmacology, evolution, and mechanism of simultaneous interactions at the receptor site in both the cabbage looper and the corn earworm.

DEVELOPMENT AND FIELD TESTING OF A PORTABLE PHEROMONE MONITORING DEVICE

M.S. Mayer, E.R. Mitchell and C.A. Litzkow

Objective: To develop a portable device that relies on the electroantennogram (EAG) to monitor and measure pheromone levels in field environments.

Methods: A portable, commercially-available high input impedance biological preamplifier has been incorporated into a proprietary mounting that supports a live insect. The insect and electrode assembly are isolated from ambient air within a chamber whose interior is purged with clean air from a portable SCUBA tank until measurements are made. The insect's antennas project over an opening through which ambient, pheromone-laden air is drawn by a small fan. The electrical potentials that comprise the EAG are monitored by electrodes connecting the eyes and the tips of the antenna. The device is connected to a battery-powered recorder. A prototype calibration device has been designed to operate in conjunction with and appropriately calibrate the recorded EAG's in terms of static airborne concentration. The prototype went through several refinements during the latter part of 1994 which enabled the assay of Z11-16:Al release in cotton field plots.

Results: Early in the experiments the device was tested at several heights within treated cotton plots. To assure that potentials monitored on the recorder were responses to sex pheromone, smoke was generated at sites corresponding to emitter sites on the cotton plant. The generated smoke demonstrated differences in emission patterns that were dependent on both the wind and row orientation. Over a period of several weeks, the EAG monitor was passed through the field plots near emitter sources. Recorded EAG potentials corresponded every time to expected emission sources. As a result of these combined smoke and EAG experiments, we concluded that the EAG deflections were, indeed, antennally detected pheromone. The problem of measuring the absolute airborne concentration at various field sites is made difficult due to the dynamic relationships of the antennal response and the structure of the stimulus plume.

Plans: The prototype EAG apparatus will be tested in a cabbage field and in a cotton field during 1995. Studies with smoke will be incorporated into the test paradigm to ensure that metered potentials indicate the actual detection of pheromone. A data logger will be incorporated into the system and digital output will be analyzed to determine whether or not the system can be used to obtain relative measures of different treatment concentration levels.

THE RELATIONSHIP BETWEEN Z7-12:AC DETECTION BY AN ANTENNAL SPECIALIST RECEPTOR NEURON AND MATING BEHAVIOR IN THE CABBAGE LOOPER MOTH

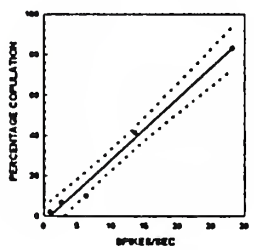
M.S. Mayer

Objective: To measure the relationship in the central nervous system that occurs between the input neurophysiological responses of antennal specialist receptor neurons and the amount of elicited behavior to adequate pheromonal stimuli.

Methods: Responses of individual antennal specialist receptor neurons were measured at the same airborne concentrations of Z7-12:Ac that were used to measure flight behaviors in a wind tunnel. Because the final airborne concentration of Z7-12:Ac was deployed at the same levels in both types of assay, it was possible to directly compare the amount of behavioral response to the receptor neuronal input at each common stimulus level.

Results: The figure shows that the average number of spikes elicited per sec at a given Z7-12:Ac airborne concentration linearly correlates with the percentage of males hairpencilling.

Conclusions: This finding shows that the amount of terminal pheromone-elicited behavior is linearly correlated with the number of action potentials evoked from the peripheral receptor neurons. It would appear that, as far as the amount of behavior elicited, there is little to no transformation of input responses. Thus, the amount of terminal sex pheromone mediated behavior is simply the sum of the response of the peripheral HS(a) receptor neurons. This may argue in favor for the reflexive nature of the response, at least in the cabbage looper.



Plans: To continue to investigate the significance of the linear relationship between the amount of behavior to the amount of peripheral receptor neuron activity.

MANIPULATION OF BACTERIAL SYMBIONTS FOR SUPPRESSION OF LEPIDOPTERA

S.G. Miller

Objectives: To identify and characterize symbiotic microorganisms from Lepidoptera, which might be manipulated for use as suppression agents in alternate insect hosts. To generate baseline information concerning the growth, replication and nutritional requirements of microorganisms in natural and alternate hosts.

Methods: Bacteria were isolated from extracts of insect tissues via cultivation on nutrient media and agar plates. Preliminary taxonomic identifications of bacteria were made through sequence analyses of 16s ribosomal RNA genes generated by the polymerase chain reaction.

Results: A bacterium that preferentially replicates in tissues of *Heliothis virescens* x *H. subflexa* backcross hybrids was identified via cultivation and subsequently determined to be an Enterococcus by PCR analyses of its 16s ribosomal RNA. The bacterium was found to occur in high titers in the larval and adult midgut, and infection was also detected (via cultivation and PCR studies) in Malpighian tubules, ovaries and testes. The Enterococcus has no discernible effect upon backcross hybrid moths, however, curing the bacterium by surface-sterilization of eggs reduces female fecundity and fertility. Introduction of the bacterium into *H. virescens* and other Noctuid moths leads to infection of the same tissues in addition to reduced fertility of both male and female adults. Unlike the backcross hybrids, transmission of the Enterococcus to the F_1 generation in other moths is poor.

Plans: continued evaluations of Enterococcal infectivity and transmission in "alternate" insect hosts are planned, including more detailed studies of gene expression in transinfected gonadal tissues. Attempts to introduce recombinant plasmids via conjugal transfer will be made with the ultimate goal of increasing transmissibility or further enhancing the bacterium's fertility-reducing capacity.

IMPACT OF *COTESIA PLUTELLAE* ON DIAMONDBACK MOTH IN CABBAGE

E.R. Mitchell, F.C. Tingle, and R.C. Navasero-Ward

Objective: To evaluate the impact of the exotic parasitoid *Cotesia plutellae* on control of diamondback moth (DBM) in commercial cabbage.

Methods: *C. plutellae* were purchased as cocoons from Biofac, Inc., Mathis, TX, flown overnight to Gainesville, FL, and released in commercial cabbage fields at Bunnell, FL, the following day. Parasitoids were released at the rate of 600 cocoons/ac in field A (30 ac), 300 adults/ac in field B (12 ac), and 300 cocoons/ac in field C (26 ac). The sex ratio of released parasitoids was ca. 50:50. Release cages measuring 22x15x12 inches high were partially covered on the sides with 1/8-in mesh hardware cloth to allow escape of adult parasitoids. Cabbage plants with feeding diamondback larvae were placed inside each cage for conditioning of parasitoids; a cotton ball soaked with a 10% solution of honey/sugar water served as a food source. The release cages were located equidistantly throughout each field.

DBM larvae and pupae were counted and collected weekly form 135 plants (78 in late season) at 9 locations in field B and 18 locations in fields A, C, and the control field. Most of the larvae collected were dissected to determine if they were parasitized,; but some were held on artificial diet for emergence to determine the accuracy of parasitoid identifications determined by dissection.

Results: In fields where *C. plutellae* cocoons were released, total diamondback larval parasitism increased 40% (field A, 600 cocoons/ac) to 67% (field C, 300 cocoons/ac) over the level of parasitism attributed to the native parasitoid *Diadegma insulare* (Table 1). However, total parasitism in field C was very low (9.9%) compared to fields A and B due to unnecessary pesticide applications that prevented the natural increase of *Diadegma* and survival of the *Cotesia* released. .In field B where 300 *Cotesia* adults/ac were released, total larval parasitism increased 15% over that caused by *Diadegma*.

The seasonal population of diamondback larvae and pupae (combined) was not significantly different among fields. Also, plant damage ratings of mature cabbage at season's end showed no significant differences among fields (Table 2).

If growers had relied upon the infestation and plant damage rating data we provided them each week, they would have saved several pesticide applications, and *C. plutellae* would have been much more effective at controlling diamondback moth infestations in the cabbage.

Plans: Parasitoid releases and mating disruption technology will be combined in the 1995 growing season to determine the impact on control of diamondback moth in cabbage.

Table 1. Seasonal mean parasitism of diamondback larvae in cabbage. Bunnell, FL. 1994

Field	No. *Cotesia plutellae*/ac[1]	Percent parasitism			
		Diadegma insulare	*Cotesia plutellae*	Total	Increase[2]
Control	0	17.2	0.0	17.2	NA
A	600	22.0	15.1	37.1	40.4
B	300	30.5	5.4	35.9	15.0
C	300	3.3	6.6	6.6	66.7

[1] 4 releases; sex ratio = 50:50

[2] Increase in parasitism over level attributed to the native parasitoid, *D. insulare*.

Table 2. Cabbage ratings (1-6) at harvest. Bunnell, FL. 1994

Field	Number of locations	Mean rating (13 plants/location)
Control	10	2.0 ± 0.10
A	9	2.0 ± 0.10
B	5	1.9 ± 0.04
C	9	1.8 ± 0.10

Rating Scale for Cabbage at Harvest

1. No damage to head, wrapper leaves, or outer leaves.
2. No damage to head or wrapper leaves; some damage on outer leaves.
3. No damage to head; some damage to wrapper leaves.
4. Minor damage to head; no feeding damage through outer head leaves.
5. Damage through outer head leaf (usually looper).
6. Head severely damaged. Cabbage not marketable.

MATING DISRUPTION TRIALS IN CABBAGE: EFFECT OF SHIN-ETSU ROPE FORMULATIONS ON MATING OF DIAMONDBACK MOTH AND CABBAGE LOOPER

E.R. Mitchell, F.C. Tingle, and R.C. Navasero-Ward

Objective: To control diamondback moth (DBM) and cabbage looper (CL) in commercial cabbage with pheromone. All tests were conducted in winter-spring 1994 at Bunnell, FL.

Methods: A total of 79 ac of cabbage was treated with Isomate-DBM pheromone. A 54 ac field was treated on Jan. 27 with pheromone (Lot 41001) that had been in cold storage since fall 1990. It was used because of delayed shipment of fresh DBM pheromone from Japan in time for the 1994 trial. A 2nd field of 25 ac, adjacent to the 54 ac, was treated with DBM pheromone (Lot 51201, received Feb. 14) on Feb. 17. A 3rd field of 30 ac was treated with a combination of DBM and CL pheromone (Lot DCM 5Y044, received Dec. 12, 1993) on Feb 25. All treatments were applied at the rate 400 yds/ac; attached to stakes 12-14 in. above ground; with each line 8-10 yds from the next. The combo (DCM 5Y044) formulation was applied at the same rate/ac. However, the combo 'rope' was cut into 8-in. pieces at the plant. Thus, 5 pieces ea were tied to survey flags, and the flags were spaced ca.11.5 ft apart throughout the field (400/ac). The ropes used for DBM only were crimped every 8 in. but were uncut. Treatment efficacy was determined by making weekly plant damage ratings and recording the number of DBM and CL larvae and pupae/plant. Mating tables with 6-8 sentinel females with one forewing clipped were used to determine if the pheromone treatment shut down mating. Two mating tables were positioned well into the field from the edge. Moths were placed in the field 1-2 h before sundown, retrieved the following morning, and dissected to determine mating status. The results were compared to sentinel females located in nearby untreated cabbage fields. Pheromone traps also were set up in each field to determine if the pheromone treatments shut down capture of wild moths in traps.

Results: The Isomate-DBM pheromone and the DBM/CL combo treatment failed to shut down mating by DBM in all fields (Table 1). Trap capture data, plant damage ratings, and larval/pupal counts (not shown) also indicated that the pheromone treatment failed to control DBM. Interestingly, fresh material (Lots 51201 and DCM 5Y044) were no more effective against DBM than pheromone stored for ca. 4 years (Lot 41001). Chemical analyses of the 3 formulations are in progress to determine if the pheromone components deteriorated over time in storage and the field. Another possible explanation for failure of the DBM pheromone to suppress mating in 1994 is that pheromone release rate was adversely affected by cooler temperatures typical of the winter and spring months. The CL pheromone suppressed mating of this pest for 47 day (Table 2). Once the problem with the DBM pheromone is resolved, it is probable that we can control both the CL and DBM by evaporating pheromones for both species from the same dispenser.

Plans: The 1994 tests will be repeated in winter-spring 1995 using fresh pheromone.

Table 1. Disruption of mating (%) by diamondback moths (DBM) in cabbage treated with pheromone. Bunnell, FL. 1994.

Date	Days PT[1]	Moths in Control		Moths in Treatment		Percent Disruption[2]
		Mated	Unmated	Mated	Unmated	
		Johnston (DBM/CL Combo)[3]				
Mar. 21	24	6	14	6	22	36.4
Mar. 29	32	17	36	22	39	0
		Emery (DBM Pheromone-1994)[4]				
Mar. 10	21	5	14	3	12	29.9
Mar. 15	26	15	19	8	16	36.6
Mar. 21	32	12	23	6	22	47.7
Mar. 29	40	13	24	12	23	3.6
		Turner and Hawkins (DBM Pheromone-1990)[5]				
Mar. 10	40	5	14	4	19	41.1
Mar. 15	45	15	19	26	30	0
Mar. 21	51	12	23	25	40	0

[1] Days after pheromone treatment was installed in field.

[2] $\frac{\text{\% mated in control - \% mated in treatment}}{\text{\% mated in control}} \times 100$

[3] Pheromones for cabbage looper & DBM were formulated in the same dispenser. Installed in field 2/25/1994.

[4] DBM pheromone was formulated in 1994 and installed in field Feb. 17, 1994.

[5] DBM pheromone was formulated in 1990 and held in cold storage until installed in field 1/27/1994.

Table 2. Disruption of mating (%) by cabbage looper moths in cabbage treated with pheromone. Bunnell, FL. 1994.

Date	Days PT[1]	Moths in Control		Moths in Treatment		Percent Disruption[2]
		Mated	Unmated	Mated	Unmated	
Mar. 21	24	13	28	0	27	100
Mar. 29	32	17	39	1	40	94.2
Apr. 05	39	26	58	2	59	92.7
Apr. 13	47	26	61	0	57	100
Apr. 19	52	2	57	1	47	40
Apr. 28	61	3	60	2	58	32

[1] Days after pheromone treatment was installed in field. The pheromone dispensers used were formulated in 1994 and contained pheromones for cabbage looper and diamondback moth.

[2] $\frac{\text{\% mated in control - \% mated in treatment}}{\text{\% mated in control}} \times 100$

SHIN-ETSU ROPE DISPENSERS FOR DISRUPTING MATING OF TOBACCO BUDWORM AND CORN EARWORM

E.R. Mitchell

Objective: To assess the efficacy and longevity of Shin-Etsu rope dispensers in disrupting mating by the tobacco budworm (TBW) and corn earworm (CEW) in small cotton plots.

Methods: Shin-Etsu rope dispensers containing a blend of Z-11-hexadecenal:Z-9-tetradecenal:Z-9-hexadecenal in a ratio of 15:1:1 (CTW 66011), 15:3:3 (CTW 66018) or 15:5:5 (CTW 66019) were tested in 0.125 ac cotton plots by tying either 1 (400/ac) or 2 (800/ac) dispensers/plant every 11.5 ft. Each dispenser (20 cm long) contained ca. 168 mg of the total pheromone blend. The treatments were installed in a N-S line, perpendicular to the prevailing wind. The distance between treatments was ca. 165 ft. The treatments were arranged in randomized blocks with 2 replications each. Separate untreated control plots were located ca. 400 yards to the west of the treated plots. Treatment effects were measured by reductions in mating of virgin sentinel female moths (6-8) placed on mating tables located in the center of each plot compared to female moths in the control areas. Virgin females with one forewing clipped were placed on tables late in the afternoon, collected the following morning, and dissected to determine mating status. Two mating tables, one for the TBW and the other for CEW, were positioned near the center of each plot. The mating tables were ca. 1 m above the ground and 4 m apart.

Results: At 400 dispensers/ac, neither 66011, 018, or 019 were effective at shutting down mating by the TBW or CEW for more than 3 weeks (Table 1). Similar results were obtained with 800 dispensers/ac of CTW 66011. These results were comparable to those obtained in a large scale field trial in which two 35 ac blocks of cotton were treated with CTW 66011 to control the TBW and CEW. However, the 15:5:5 blend (CTW 66019) at 800 dispensers/ac was near perfect at shutting down mating of both species for 5 weeks. These results indicate that 66019 is the formulation of choice to disrupt mating of TBW and CEW. However, the release rate of the dispensers must be increased significantly; or 800 dispensers/ac must be applied to achieve the desired level of near 100% shutdown of mating for an extended period.

Plans: Small plot trials will be conducted to evaluate the efficacy and longevity of potential mating disruptants formulations for TBW and CEW.

Table 1. Mating disruption (%) of tobacco budworm and corn earworm in 0.125 ac cotton plots treated with Shin-Etsu formulations numbers 66011, 018, and 019 using 400 or 800 rope dispensers/ac. Trenton, FL. 1994.

Date	66011 400	66011 800	66018 400	66019 400	66019 800	Mean moths per trap night
			Tobacco budworm			
Aug. 03	86.8[1]		91.2[1]	70.0[1,2]		
Aug. 11	70.1		36.3	61.6		165.3
Aug. 17	60.7		70.2	91.6		38.7
Aug. 24	88.3[3]	89.6[3]		100	100[3]	1.8
Sep. 01	76.4	82.3		100	100	15.8
Sep. 07	89.3	82.9		51.2	100	15.7
Sep. 14	27.9	44.7		35.9	100	29.8
Sep. 21	72.4	63.2		57.1	100	15.5
			Corn earworm			
Aug. 03	66.6[1]		26.6[1]	53.8[1,2]		43.7
Aug. 11	73.3		37.7	59.5		43.6
Aug. 17	13.1		0	0		28.0
Aug. 24	100[3]	100[3]		64.2	100[3]	6.0
Sep. 01	100	100		100	100	46.2
Sep. 07	52.8	89.1		100	100	43.2
Sep. 14	0	49.6		22.2	74	25.5
Sep. 21	80.2	100		26.3	100	22.5

[1] Test set out on July 26 and terminated Aug. 17.
[2] The dispensers set out July 26 were used throughout the test period.
[3] New dispensers were set out Aug. 17.

LARGE-SCALE FIELD TEST OF SHIN-ETSU PHEROMONE MATING DISRUPTANT FORMULATION FOR CONTROL OF CORN EARWORM IN COTTON

E.R. Mitchell, F.C. Tingle, and M. Kehat[1]

Objective: To control the corn earworm (CEW) in cotton by disrupting mating using excessive quantities of synthetic sex pheromone evaporated from Shin-Etsu 'rope' dispensers. All tests were conducted in dry land cotton located near Houston in northeast Suwannee County, FL.

Methods: Two 37 ac plots of cotton were treated with Shin-Etsu rope dispensers containing a 15:1:1 blend of Z11-hexadecenal:Z9-tetradecenal:Z9-hexadecenal (Lot CTW 66011). Each rope was 8-in. long and contained ca. 160 mg total pheromone blend. Two ropes/plant were tied 8-10 in. above the soil, spaced ca. 17.3 ft apart throughout the field for a total of 400/ac. The ropes were applied when the plants were in the 8-10 leaf stage (field A, July 12-14; field B, July 7-8). Another cotton field of 35 ac planted ca. the same time as fields A and B was used as a control. Field A was ca. 0.125 mi from the control field and 2.5 mi from field B. The nearest cotton to field B was ca. 1.0 mi to the north. The 3 test sites were selected because of similarities in planting date, size, and shape.

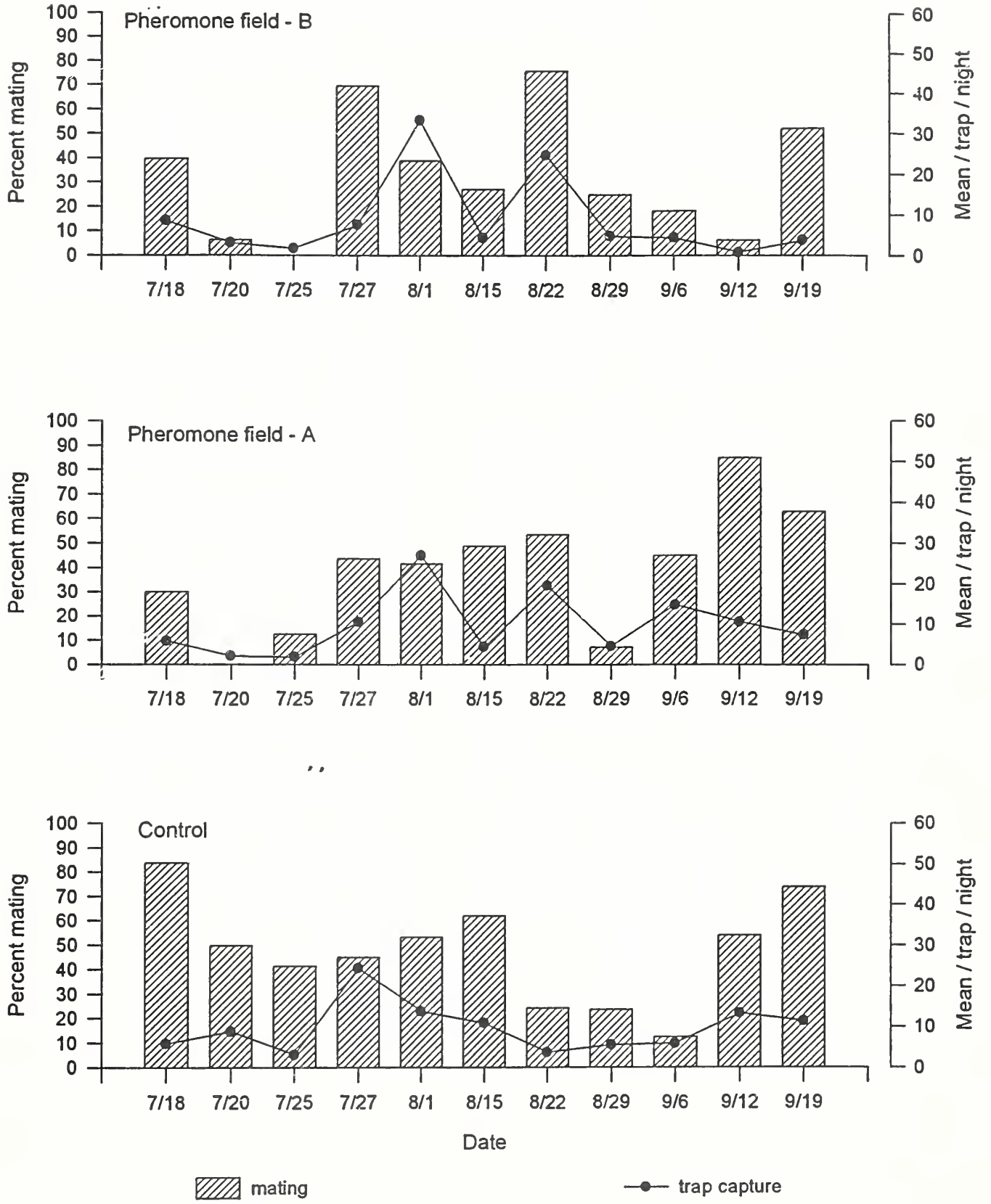
Treatment efficacy was measured by shutdown of male moths captured in wire cone traps baited with CEW sex pheromone (2 ea/field), reduction in matings by sentinel females (6-8) positioned on mating tables (2 ea/field), and weekly counts of bollworm eggs and larvae in each field versus the control field. Pheromone traps and mating tables were positioned 1/3 of the way in from each corner of the field; mating tables were located diagonally across the field from the pheromone traps. This allowed for the continuous operation of pheromone traps, even on the nights that sentinel females were set out on mating tables.

Results: The Shin-Etsu rope formulation was ineffective at shutting down mating by the CEW (Fig. 1). Capture of CEW males in pheromone traps were not excessive compared to past seasons when the 15:1:1 blend performed well in small plot tests. Results of small plot trials in cotton this past summer using different numbers of rope dispensers/ac (400, 800, 1600, and 2400) showed that the level of mating disruption was proportional to the number of ropes applied, i.e., the more ropes used/ac the greater the level of mating disruption. Thus, the poor performance of the Shin-Etsu ropes against CEW this season appeared to be due, at least in part, to pheromone release rate; 400 ropes/ac simply did not release enough pheromone to effectively shut down mating by the CEW. Possible solutions to enhanced effectiveness against the CEW include: drastically increase the number of dispensers/ac of the present formulation; increase the release rate of the present dispenser; or use a different formulation (see Shin-Etsu Rope Dispensers....).

Plans: Mating disruption trials for CEW will continue in the 1995 season.

[1] The Volcani Center, Bet Dagan, Israel

Fig. 1 Mating of corn earworm in cotton treated with Shin-Etsu mating disruptant pheromone evaporated from rope dispensers.



LARGE-SCALE FIELD TEST OF SHIN-ETSU PHEROMONE MATING DISRUPTANT FORMULATION FOR CONTROL OF TOBACCO BUDWORM IN COTTON

E.R. Mitchell. F.C. Tingle, and M. Kehat[1]

Objective: To control the tobacco budworm (TBW) in cotton by disrupting mating using excessive quantities of synthetic sex pheromone evaporated from Shin-Etsu 'rope' dispensers. All tests were conducted in dry land cotton located near Houston in northeast Suwannee County, FL.

Methods: Two 37 ac plots of cotton were treated with Shin-Etsu rope dispensers containing a 15:1:1 blend of Z11-hexadecenal:Z9-tetradecenal:Z9-hexadecenal (Lot CTW 66011). Each rope was 8-in. long and contained ca. 160 mg total pheromone blend. Two ropes/plant were tied 8-10 in. above the soil, spaced ca. 17.3 ft apart throughout the field for a total of 400/ac. The ropes were applied when the plants were in the 8-10 leaf stage (field A, July 12-14; field B, July 7-8). Another cotton field of 35 ac planted ca. the same time as fields A and B was used as a control. Field A was ca. 0.125 mi from the control field and 2.5 mi from field B. The nearest cotton to field B was ca. 1.0 mi to the north. The 3 test sites were selected because of similarities in planting date, size, and shape.

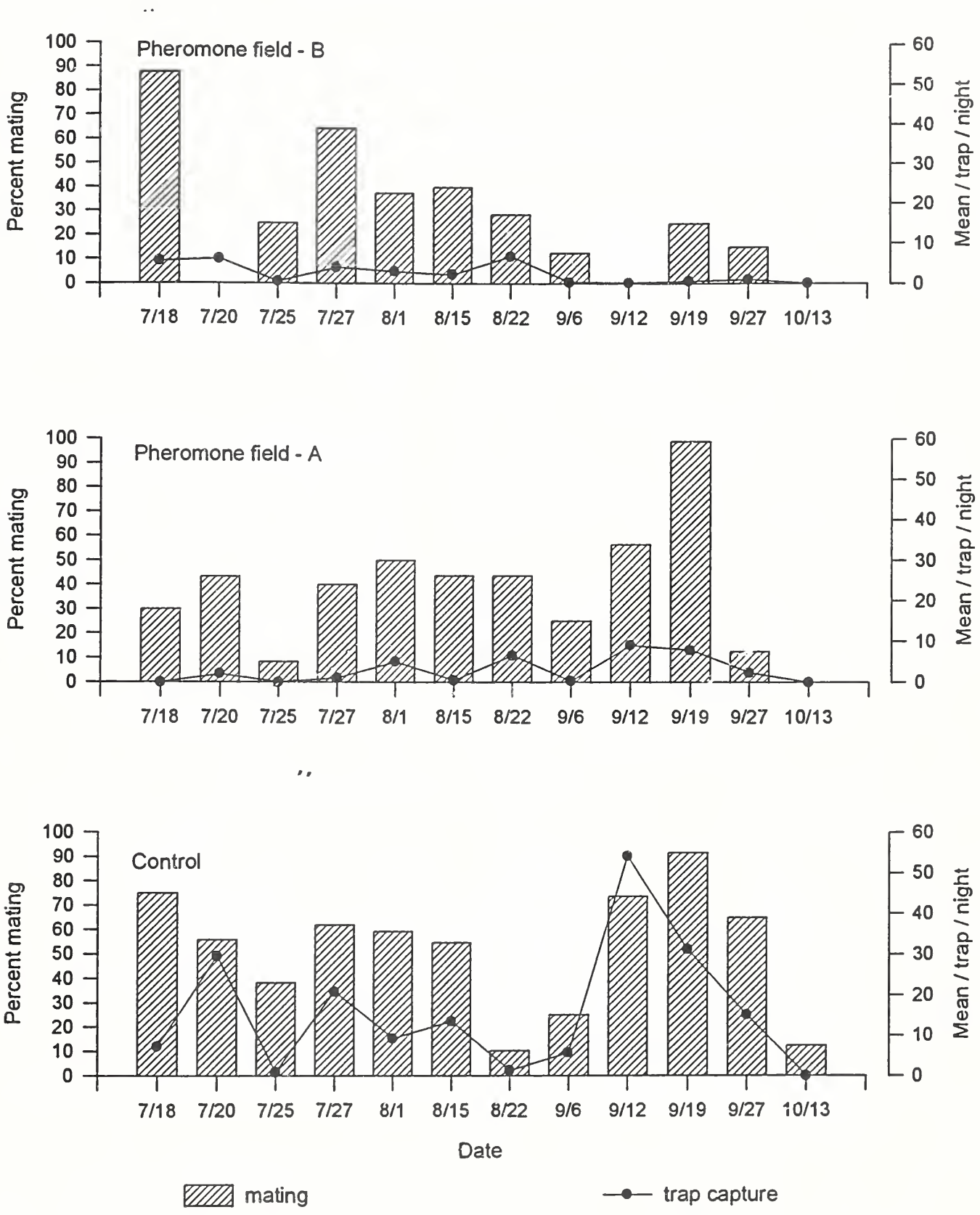
Treatment efficacy was measured by shutdown of male moths captured in wire cone traps baited with TBW sex pheromone (2 ea/field), reduction in matings by sentinel females (6-8) positioned on mating tables (2 ea/field), and weekly counts of bollworm eggs and larvae in each field versus the control field. Pheromone traps and mating tables were positioned 1/3 of the way in from each corner of the field; mating tables were located diagonally across the field from the pheromone traps. This allowed for the continuous operation of pheromone traps, even on the nights that sentinel females were set out on mating tables.

Results: The Shin-Etsu rope formulation was ineffective at shutting down mating by the TBW (Fig. 1). Capture of TBW males in pheromone traps were not excessive compared to past seasons when the 15:1:1 blend performed well in small plot tests. Small plot trials in cotton this past summer using different numbers of rope dispensers/ac (400, 800, 1600, and 2400) showed that the level of mating disruption was proportional to the number of ropes applied, i.e., the more ropes used/ac the greater the level of mating disruption. Thus, the poor performance of the Shin-Etsu ropes against TBW this season appeared to be due, at least in part, to pheromone release rate; 400 ropes/ac simply did not release enough pheromone to effectively shut down mating by TBW. Possible solutions to enhanced effectiveness against the TBW include: drastically increase the number of dispensers/ac of the present formulation; increase the release rate of the present dispenser; or use a different formulation (see Shin-Etsu Rope Dispensers....).

Plans: Mating disruption trials for TBW will continue in the 1995 season.

[1] The Volcani Center, Bet Dagan, Israel

Fig. 1 Mating of tobacco budworm in cotton treated with Shin-Etsu mating disruptant pheromone evaporated from rope dispensers.



SUPPRESSION OF BEET ARMYWORM IN COTTON WITH PHEROMONE

E.R. Mitchell, F.C. Tingle, and M. Kehat[1]

Objective: To control beet armyworm in cotton by disrupting mating using excessive quantities of synthetic sex pheromone evaporated from Shin-Etsu 'rope' dispensers. All tests were conducted in dry land cotton located near Houston in northeast Suwannee County, FL.

Methods: Two 37 ac plots of cotton were treated with Shin-Etsu rope dispensers containing a 70:30 blend of Z9,E12-tetradecadien-1-ol acetate and Z9-tetradecen-1-ol (Lot 65010). Each rope was 8-in. long and contained ca. 160 mg total pheromone blend. Two ropes/plant were tied 8-10 in. above the soil, spaced ca. 17.3 ft apart throughout the field for a total of 400/ac. The ropes were applied when the plants were in the 8-10 leaf stage (field A, July 12-14; field B, July 7-8). Another cotton field of 35 ac planted ca. the same time as fields A and B was used as a control. Field A was ca. 0.125 mi from the control field and 2.5 mi from field B. The nearest cotton to field B was ca. 1.0 mi to the north. The 3 test sites were selected because of similarities in planting date, size, and shape.

Treatment efficacy was measured by shutdown of male moths captured in Universal Moth traps baited with BAW sex pheromone (2/field), reduction in matings by sentinel females (6-8) positioned on mating tables (2/field), and weekly counts of beet armyworm egg masses and larvae in each field versus the control field. Pheromone traps and mating tables were positioned 1/3 of the way in from each corner of the field; mating tables were located diagonally across the field from the pheromone traps. This allowed for the continuous operation of pheromone traps, even on the nights that sentinel females were set out on mating tables.

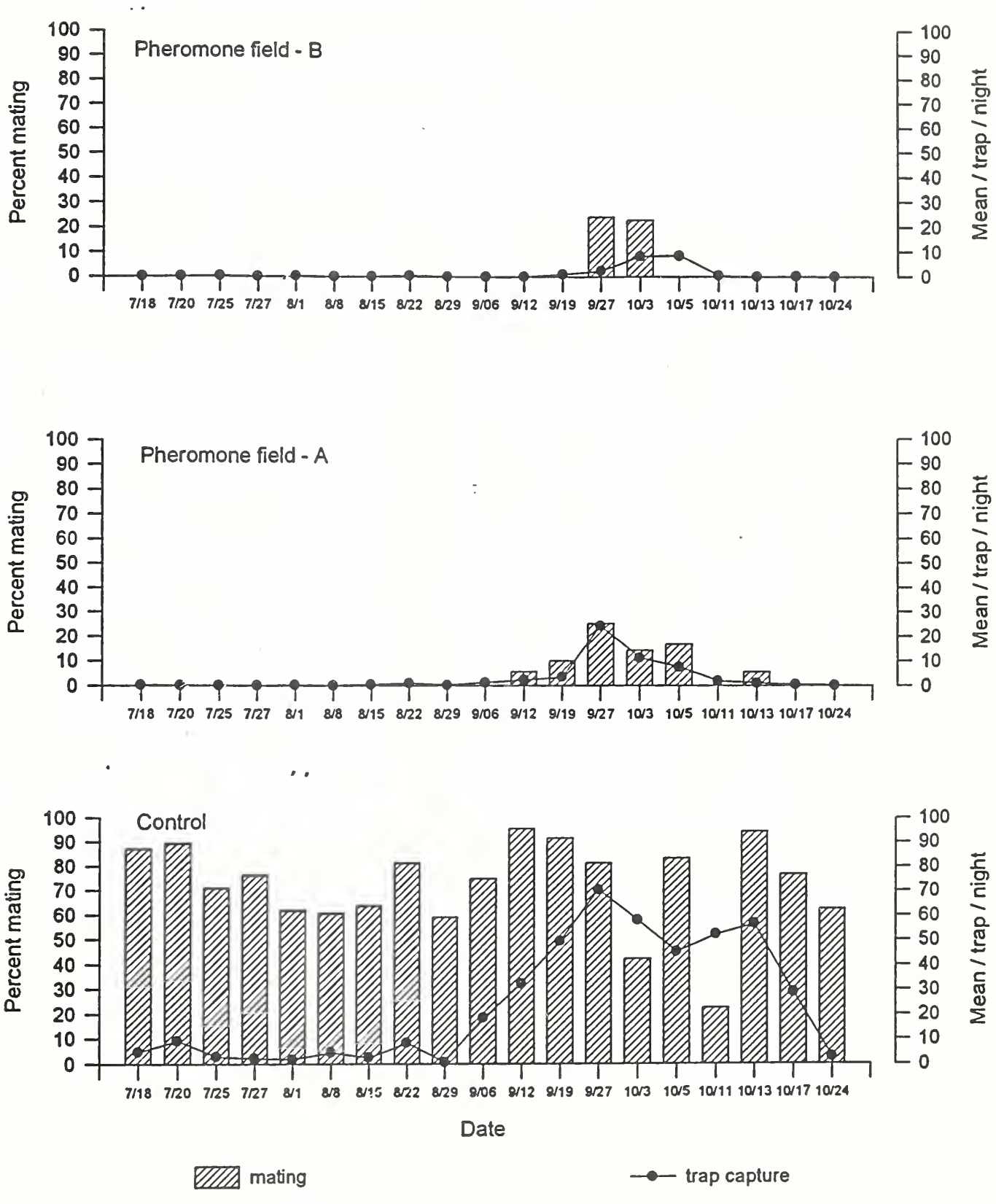
Results: Shut down of mating by BAW was almost complete throughout the entire growing season (Fig. 1). The BAW population was low initially as shown by trap capture data , but began to increase rapidly by the end of August. However, the level of mating in the pheromone-treated fields showed only a slight increase during September and October when the BAW population exploded, averaging >70 males/trap on September 27. The number of BAW eggs and larvae recorded in the pheromone treatments also showed significant reductions over the control field

Table 1. BAW larvae per plant. 1994. Means in the same column followed by different letters are significantly different, P < 0.004, SNK Test.

Field	Sept. 26	Oct. 05
Control	0.62 a	0.13 a
Pheromone A	0.06 b	0 b
Pheromone B	0 b	0.01 b

Plans: A manuscript on this phase of work is in preparation. This will be repeated in 1995 using a combination of BAW and fall armyworm pheromones in separate rope dispensers.

Fig. 3 Mating of beet armyworm in cotton treated with Shin-Etsu mating disruptant pheromone evaporated from rope dispensers.



IMPACT OF PHEROMONE DOSAGE ON SHUTDOWN OF MATING IN TOBACCO BUDWORM AND CORN EARWORM

E.R. Mitchell

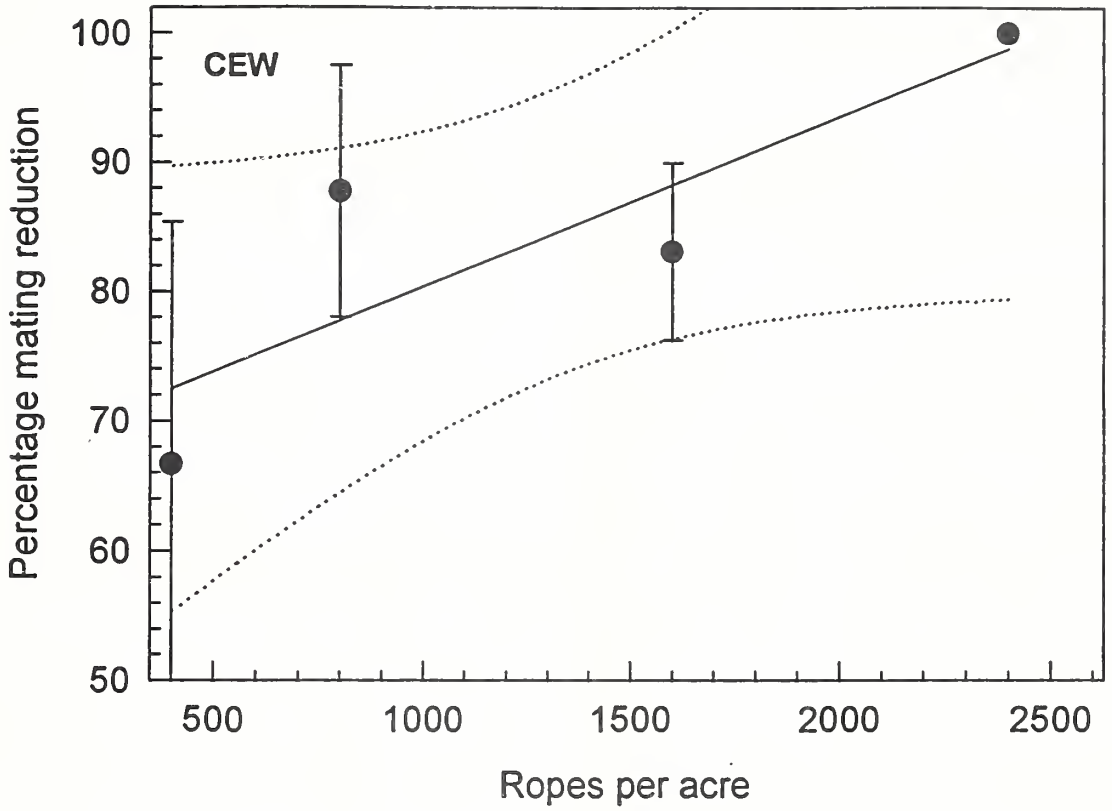
Objective: To determine effect of different numbers of Shin-Etsu rope dispensers/ac on mating by the tobacco budworm (TBW) and corn earworm (CEW). Results of small plot and large field trials in 1994 suggested that the erratic results on shutdown of mating in TBW and CEW using 400 ropes/ac possibly were due to the fact that the pheromone release rate/dispenser simply was too low to consistently provide near 100% mating disruption over an extended period.

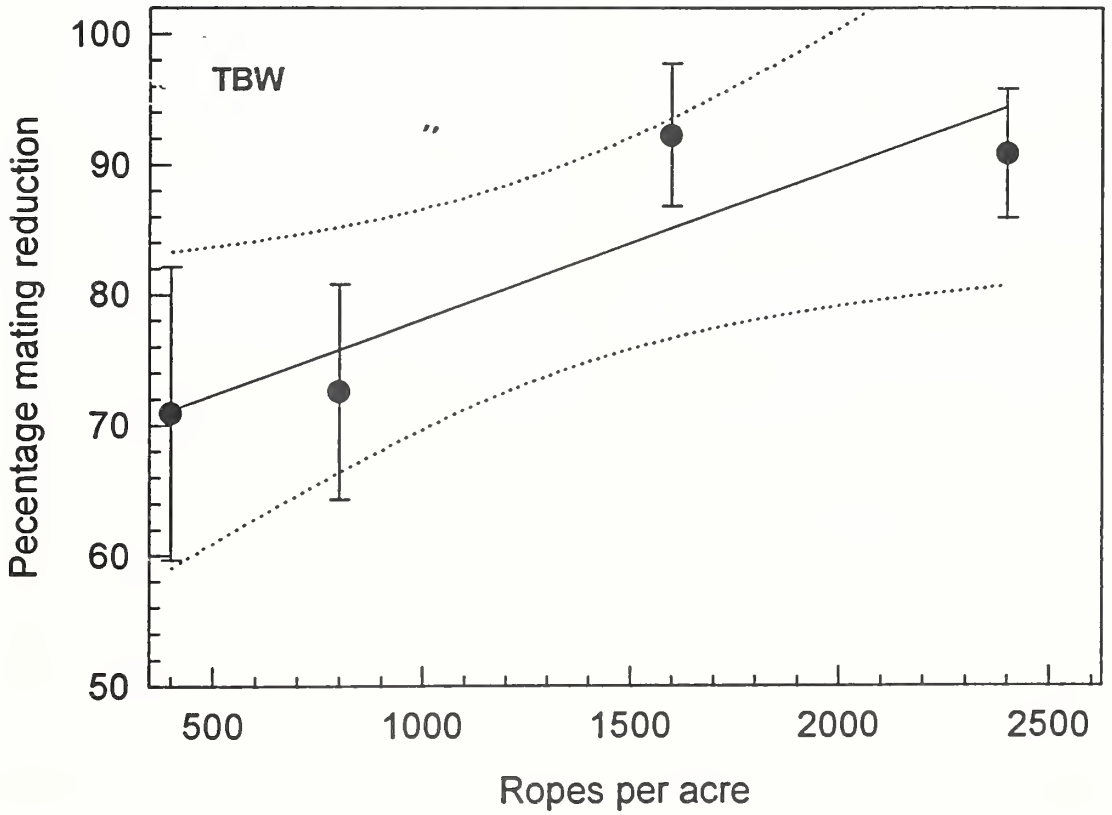
Methods: Shin-Etsu rope dispensers containing a 15:1:1 blend of Z-11-hexadecenal:Z-9-tetradecenal:Z-9-hexadecenal in a ratio of 15:1:1 (CTW 66011) was tested in 0.125 ac cotton plots by tying either 1 (400/ac) or 2 (800/ac), 4 (1600), or 6 (2400) dispensers/plant every 11.5 ft. Each dispenser (20 cm long) contained ca. 168 mg of the total pheromone blend. The treatments were installed in a N-S line, perpendicular to the prevailing wind.. The distance between treatments was ca. 165 ft. The treatments were arranged in randomized blocks with 2 replications each. Separate untreated control plots were located ca. 400 yards to the west of the treated plots. Treatment effects were measured by reductions in mating of virgin sentinel female moths (6-8) placed on mating tables located in the center of each plot compared to female moths in the control areas. Virgin females with one forewing clipped were placed on tables late in the afternoon, collected the following morning, and dissected to determine mating status. Two mating tables,one for the TBW and the other for CEW, were positioned near the center of each plot. The mating tables were ca. 1 m above the ground and 4 m apart.

Results: There clearly was a dose response effect on the level of mating by both the TBW and CEW with the highest level of mating disruption achieved with 2,400 ropes/ac (Figure 1). ANOVA showed there was no significant interaction between dates over the test period, July 26-Aug. 17. Mean number of moths captured per trap night during the test period was: CEW, 28.6; TBW, 15.7. These results suggest that the level of mating disruption for both species can be significantly improved by (1) increasing the release rate of individual dispensers and continue to use 400 units/ac or (2) increase the number of CTW 66011 ropes (releasing pheromone at current rate) to 2400/ac or more. A third alternative would be to use 800 ropes/ac of CTW 66019 (see Shin-Etsu Rope Dispensers...). Possibly the best alternative would be to increase the release rate of CTW 66019 and use 400 dispensers/ac.

Plans: Small plot trials will be conducted to evaluate the efficacy and longevity of potential mating disruptants formulations for TBW and CEW.

Fig. 1. Mating reduction of corn earworm (CEW) and tobacco budworm (TBW) using 400-2400 Shin Etsu ropes (#66011) per acre. Trenton, FL. 1994.





ESTIMATION OF MATING DISRUPTION: COMPARISON OF FEMALE MOTHS TETHERED INDIVIDUALLY VERSUS CLIPPED-WINGED FEMALES ON MATING TABLES

F. Mochizuki[1] and E.R. Mitchell

Objective: To determine the mating efficacy of female tobacco budworm (TBW)and corn earworm (CEW) moths tethered (TF) individually on a string vs. a 'clump' of 6-8 clipped-winged females on a mating table (MT).

Methods: Two to 4-day old females were used in all experiments. Moths were tethered by tying a cotton thread around the right forewing. Six TF were tied ca. 1 m above ground to dowels spaced ca. 1.8 m apart in a line. TF were positioned at least 8 m away from a MT. Six to 8 females with the right forewing clipped to prevent escape were positioned on a MT (white, 52 cm long x 39 cm wide x 8 cm deep) positioned ca. 1 m above the ground. All trials were carried out in cotton that was 1-1.5 m high. The insects were placed in the field 1-2 hr before sunset, collected the following morning, and returned to the laboratory for dissection to determine mating status. A competitive experiment was conducted to determine if male moths showed a preference for 'normal', unclipped females compared to tethered or clipped-wing females. Two, 2- to 4-day old females (unclipped, clipped-wing, or tethered,) were placed in a 30 x 30 x 30 cm screened cage with a single male moth of similar age. The moths were held outside overnight under a protected shelter, and dissected the following morning to determine mating status. Sixteen cages were used simultaneously, and a cotton ball immersed in honey/sucrose solution was set in each cage served as a food source. Data were subjected to Chi-square analysis.

Results: It took 1.0-1.5 min and 10-15 min to prepare the MT females and the TF, respectively (Table 1). Preparation of clipped-wing females for MT was much faster, ca. 10X, than tying a thread around the forewing of the same number of moths used for tethering. The installation females on MT also was faster than for tethering moths requiring ca. 5 and 15 min, respectively. Collection times for both methods required ca. 2-3 min each.

The collection and mating of TF and clipped-wing moths on MT in a cotton treated with pheromone and cotton without pheromone are shown in Table 2. The number of TF's retrieved was less than the number of females retrieved from MT's in only one field, primarily due to predation by spiders and possibly birds. The percentage of moths mating showed the same tendency in pheromone-treated and untreated cotton. Male moths were just as likely to mate with tethered or clipped-wing moths as with 'normal' moths (Table 3). Thus, there was no negative effect on a moth's mating ability because one wing was tied with a string or clipped to prevent flight. Also, there was no significant difference in mating efficacy of moths 'clumped' on a MT or tethered singly on a wooden pole.

Plans: A manuscript reporting these results is in preparation.

[1] Shin-Etsu Chemical Co., Ltd., Tokyo, Japan

Table 1. Estimated minutes required for preparation, installation and collection of ten females for the mating table method (MT) and the tethered female method (TF).

	MT	TF
Preparation	Clipping wings with scissors 1.0 - 1.5 min	Tying wings with a string 10 - 15 min
Installation	Putting females on MT in the field 2 - 3 min	Tethering to stakes in the field 10 - 15 min
Collection	Collecting moths in the morning 2 - 3 min	Collecting moths in the morning 2 - 3 min

Table 2. Retrieval and mating of the tobacco budworm (TBW) and the corn earworm (CEW) using the mating table method (MT) and the tethered female method (TF). Trenton, FL. 1994.

Species		The number of females Installed	Retrieved (%)		Mated (%)	
8/4-5 - No pheromone						
TBW	MT	18	17	(94%)	16	(94%)
	TF	18	13	(72%)	12	(92%)
CEW	MT	18	18	(100%)	15	(83%)
	TF	18	12	(67%)	8	(67%)
8/11-12 - No pheromone						
TBW	MT	16	13	(81%)	11	(85%)
	TF	12	6	(50%)	6	(100%)
CEW	MT	16	15	(94%)	12	(80%)
	TF	12	5	(42%)	4	(80%)
8/11-12 - Pheromone field						
TBW	MT	16	12	(75%)	3	(25%)
	TF	12	11	(92%)	2	(18%)
CEW	MT	16	14	(88%)	3	(21%)
	TF	12	11	(92%)	2	(18%)

Table 3. Comparison of mating efficacy among clipped-wing, tied wing, and control tobacco budworm (TBW) and corn earworm (CEW) females.

Species	Number of cages with					
	n	Clipped-wing female mated	Tied-wing female mated	Control female mated	Unmated	$X^2(v=1)$
TBW	16	7	9	-	0	0.25NS
	16	9	-	6	1	0.60NS
	16	-	8	8	0	0.00NS
CEW	16	8	5	-	3	0.69NS
	16	8	-	8	0	0.00NS
	16	-	8	7	1	0.07NS

SYSTEMIC RELEASE OF CATERPILLAR INDUCED COMPOUNDS FROM UNDAMAGED COTTON LEAVES

U.S.R. Röse, A.T. Proveaux, and J.H. Tumlinson

Objective: 1. To investigate whether there are volatiles released from undamaged top leaves of a cotton plant that is damaged by caterpillars on the lower leaves only 2. To determine how long the induction of volatiles takes and identify the released compounds 3. To study the effect of artificial damage of systemically induced leaves.

Method: In all experiments starved third-instar larvae of beet armyworm, *Spodoptera exigua*, were caged and replaced each day on the lower leaves of a cotton plant (8 leaves), *Gossypium hirsutum*, var. "Delta Pine 90". The undamaged top leaves of the damaged plant were enclosed in a glass sleeve of the volatile collection chamber developed by R.R. Heath and A. Manukian. Volatiles were collected from the top leaves of the systemic cotton and top leaves of a control for four days, each day from 1200-1500 hours and from 1500-1800 hours, the time of maximum release of induced compounds in cotton. On the fifth day the top leaves of the systemic and the control plant were cut off and both artificially damaged with a razor blade, immediately prior to placing them in two separate volatile collection chambers in the laboratory. Volatiles were collected from 1200-1500 on volatile collection filter, containing 25mg Super Q as an adsorbent. Volatiles were extracted from the filters by washing them with Methylene Chloride. Extracts were analyzed by capillary gas chromatography and GC-mass spectroscopy.

Results: Only after the second day small amounts of volatiles could be collected from the undamaged leaves of the caterpillar damaged cotton plant. On day three distinct amounts of volatiles were released and showed a clear systemic response of cotton to caterpillar damage. All of the compounds released, were compounds that are known to be induced by caterpillar damage, when volatiles were collected from a whole damaged plant (J.H. Loughrin 1994). Compounds collected from the systemically induced cotton were: Z-3-hexenyl acetate, E-ß-ocimene, linalool, (E)-2,4-dimethyl 1,3,7-nonatriene, (E)-ß-farnesene, (E,E)-α-farnesene, (E,E)-4,8,12-trimethyl 1,3,7,11 tridecatetraene. Control plants released small amounts of nonatriene and tridecatetraene, probably due to some plant stress experienced in the glass collection chamber. In addition, isomeric hexenyl butyrates and 2-methylbutyrates were released from the systemically induced cotton, when artificially damaged with a razor blade, but not from a control plant. Indole that can be found in large amounts when volatiles are collected from the whole damaged plant, could only be found in trace amounts, even after artificial damage.

Plans: Volatiles released from caterpillar damaged cotton leaves only, will be compared to the volatiles obtained from undamaged leaves of the systemically induced cotton. The study of the attraction of parasitoids to the systemic compounds in cotton will be continued.

EFFECT OF FRASS VOLATILES ON THE ABILITY OF THE SPECIALIST PARASITIC WASP *MICROPLITIS CROCEIPES* TO DIFFERENTIATE BETWEEN ITS HOST *HELICOVERPA ZEA*, AND NON-HOST LARVAE IN THE WIND TUNNEL.

U.S.R. Röse, M. Rutledge, J. Lockerman, W. J. Lewis, and J.H. Tumlinson

Objective: 1. To determine whether frass volatiles from different lepidoptera larvae species release different volatiles 2. To investigate whether parasitic wasps can distinguish between frass from different caterpillars in the wind tunnel, when given a choice between host and non host frass.

Method: In all experiments caterpillar frass was collected from 3rd instar lepidoptera larvae, previously fed on cotton plants, *Gossypium hirsutum*, nectaried var. "Delta Pine 90" for two days. The frass used for volatile collection and for wind tunnel experiments was less than 3 hours old. For volatile collections 100mg frass from the host larvae, *Helicoverpa zea* (CEW), and the non host larvae *Spodoptera exigua* (BAW), *Spodoptera frugiperda* (FAW), and *Trichoplusia ni* (CL) were placed in a volatile collection chamber and volatiles were collected for 3 hours on filters with super-Q adsorbent. Volatiles were extracted with Methylene Chloride and analyzed by capillary gas chromatography. Wind tunnel experiments were conducted as two choice experiments, each experiment comparing fresh host (H. zea) and non host (*S. frugiperda*, *S. exigua*)frass as a volatile source. Wasps experienced on host frass obtained from cotton fed *H. zea* larvae were released 80 cm downwind of the volatile sources, and given three chances to complete a flight to either source.

Results: Only small amounts of volatiles were collected from caterpillar frass, presumably most compounds also released from damaged cotton plants. It appears that CEW-frass contains a compound not prevalent in non host frass. Wind tunnel experiments showed the ability of *M. croceipes* to distinguish between host frass (CEW) and non host frass (BAW or FAW). Given a choice between CEW and BAW, about 75% (median) of the wasps that showed a complete flight choose CEW, while 25% (median) flew to BAW frass. A similar choice was observed for CEW frass (77%) vs. FAW frass (23%). This clearly shows that CEW contains a host specific, volatile compound, that enables the specialist parasitoid to recognize its host on cotton.

Plans: To identify the volatiles that enable the parasitoid to make a choice in the wind tunnel.

THE EFFECT OF EXTRAFLORAL NECTAR (EFN) ON SURVIVAL AND FECUNDITY

U.S.R. Röse, W.J. Lewis, and J.H. Tumlinson

Objective: To determine whether parasitoids can survive and reproduce with only the nectar from EFN as a food source.

Method: For all experiments potted cotton plants, *Gossypium hirsutum*, nectaried var. "Delta Pine 90" and nectariless var. "Stoneville 825" (8 leaves/2plants per pot) were reared in the greenhouse. Longevity studies were carried out in plexiglass cages 35cm x 35cm x 35cm in the laboratory under light cycle conditions of 14L:10D, temperature 23°± 2°C, and relative humidity of 60%±5% RH. For each treatment, two mated females of *Microplitis croceipes* were caged with 8 cotton plants (4 pots). The different treatments were EFN cotton, nectariless cotton (Stoneville 825), nectariless cotton with honey provided on a petri dish, and a cotton ball with distilled water only. All treatments were provided with additional distilled water on a cotton ball in a petri dish. Mortality of the parasitoids was recorded daily. For fecundity experiments, mated wasps were allowed to parasitize 30 *Helicoverpa zea* 3rd instar larvae within 2 hours, after being fed on honey provided in a petri dish with a nectariless cotton plant, or fed on EFN for 3, 6, and 9 days. Numbers of parasitized larvae, parasitoid cocoons, sex of emerged parasitoids, mortality of *H. zea* larvae and unhatched parasitoid cocoons were recorded.

Results: Extrafloral nectar appeared to sustain the parasitoids as long as honey provided on a nectariless plant. Parasitoids receiving neither honey nor extrafloral nectar, lived significantly shorter. The fecundity of mated wasps parasitizing *H. zea* larvae over 9 days indicated little difference between female parasitoids fed on honey and females fed on extrafloral nectar. Percentages of parasitoid cocoons per parasitized larvae, and percentage of parasitoids emerging from those cocoons was comparable in both treatments.

Plans: The overall goal is to determine the role and importance of EFN in increasing the presence of parasitoids in an area and facilitating their search for hosts.

THE INFLUENCE OF EXTRAFLORAL NECTARIES (EFN) ON PARASITOID FORAGING FOR PLANT PESTS.

U.S.R. Röse, W.J. Lewis, and J.H. Tumlinson

Objective: 1. To investigate whether parasitic wasps can distinguish between nectariless and extrafloral nectaried plants. 2. To study whether there is any odor associated with the extrafloral nectar itself or with the morphological structure that may facilitate the parasitoid finding the EFN.

Method: For all experiments potted cotton plants, *Gossypium hirsutum*, nectaried var. "Delta Pine 90" and nectariless var."Stoneville 825" (8 leaves/2plants per pot) were reared in the greenhouse. For wind tunnel experiments starved female parasitoids were experienced 3 times for 5 min. on either the extrafloral nectaried plant, on a nectariless plant, or with sucrose 3 times for 10 sec. in a petri dish, and compared to naive wasps with no experience at all. The wasps were then given a choice in the wind tunnel between a nectaried and a nectariless plant. Each wasp was given 3 chances to reach a plant in a direct flight from 1 m distance. For close range behavior bioassays studying the attraction of the wasps to the extrafloral nectar only, a flower model developed by J. Patt was used. Small teflon rings were glued on a petri dish in a circle to simulate nectaries. In three different experiments, two of the rings on opposite sides were filled with either honey, EFN collected from the plant, or sucrose. After experience on EFN, honey or sucrose, the wasps were released in the middle of the petri dish arena, and the frequency of touching the teflon rings with different contents were recorded, and the time elapsed till the wasps found the "filled" teflon ring.

Results: In two choice wind tunnel tests, wasps experienced on EFN showed clearly more completed flights (71%), than wasps experienced on nectariless cotton (11%) or sucrose (3%). Also those wasps experienced on EFN chose the EFN plant more frequently (68%) than those experienced on the nectariless plant (31%), or sucrose (1 of 36), which shows that the wasps are able to differentiate between the two plant varieties. In the close range experiment, wasps found EFN in teflon rings as fast as they found honey, which is known to have a distinct odor. They did however, seldomly find sucrose when released in the arena. This indicates that there is an odor to EFN that the parasitoids can use to find the nectar.

Plans: The overall goal: To determine the role and importance of EFN in increasing the presence of parasitoids in an area and facilitating their search for hosts 2. To determine how the wasps find EFN on plants, identify factors that mediate their searching behavior, and determine whether EFN increases the probability that a parasitoid will be retained on a plant, will be studied.

BENEFICIAL INSECT COUNTING AND PACKAGING (BICAP)

D. Shuman and J. Coffelt

Objective: To develop and evaluate an automated system that presents and fills containers with user selectable numbers of insects. This cooperative project was requested for use at The Land at EPCOT (Orlando, FL) which is pursuing biological control of Leafminers (*Liriomyza sativae*) as part of its Integrated Pest Management Program. The reared parasitoid (*Opius dissitus)* eggs are laid in leafminer larvae that drop off of leaves and had been manually counted, packaged, and distributed to achieve desired release rates. The automated BICAP system eliminates tedious labor, provides temporal rearing data, and can allow for expansion of their bio-control program.

Methods: The BICAP system is based on a modified computer-based Electronic Grain Probe Insect Counter (EGPIC) system. A large funnel directs the parasitized larvae dropping off leaves through a custom designed sensor head with infrared electronics to count them as they fall into a cup. A ring of cups is mounted on a turntable. Under software control, when a cup has received the user selected number of insects, the computer rotates the turntable to start filling the next cup. The system also creates a data base of the time-stamped insect counts to be used for temporal pattern analysis.

Results: The first implementation built for EPCOT operates two independent turntables and provides for software selection of number and size of cups, and data backup and system self-test intervals. Several data display options are available. Laboratory tests showed > 90% cup count accuracy and sources of error were due to multiple insects falling in a clump, insects falling during turntable rotation (these are recorded in the data base), and insects grabbing hold in the path of the sensor infrared beam. The BICAP system is presently being used to package 13,000 parasitoids per week for release in food production greenhouses at The Land and in floral gardens throughout Disney World, and the system is part of a tour at The Land exhibit at EPCOT. A patent is pending on the BICAP system.

Plans: A CRADA is being developed with Disney World aimed at upscaling the capacity of the BICAP system presently in use at The Land to operate seven independent turntables. In addition, insect grabbing problems could be reduced by incorporating the sensor head newly designed for the EGPIC system. Another improvement being considered is a vibrating presentation system to break up clumps of insects before they enter the sensor head.

EFFECT OF HYBRIDIZATION BETWEEN *HELIOTHIS VIRESCENS* AND *HELIOTHIS SUBFLEXA* ON THE RATIO OF PHEROMONE COMPONENTS PRODUCED BY FEMALES.

P.E.A. Teal

Objectives: To determine the genetic mechanisms which regulate the production of blends of sex pheromone components produced by females obtained from interspecific hybridization between *Heliothis virescens* and *H. subflexa*.

Methods: Reciprocal F_1 hybrids were obtained from crosses between *H. virescens* and *H. subflexa*. Backcross insects were obtained by crossing females of each hybrid line with males of the parent species. Adult females were injected with pheromone biosynthesis activating neuropeptide during the 7th h of the 3rd photophase after adult emergence and allowed to incubate for 1 h prior to excision of the sex pheromone gland and extraction of the gland in hexane containing internal standards to allow for quantitation of the amounts and ratios of pheromone components in the extracts. Extracts were analyzed by capillary gas chromatography using both polar and apolar phase capillary columns. Data obtained on the types of compounds, their amounts and ratios from analysis of extracts of hybrid and backcross females were compared with similar data obtained from analysis of extracts obtained from the pheromone glands of the parent species.

Results: Analysis of extracts obtained from the reciprocal hybrid females (n=125 females, each line) showed that the ratios and amounts of pheromone produced were identical. Although the ratios were more similar to *H. virescens* that to *H. subflexa*, ratios of (Z)-9-hexadecenal and (Z)-11-hexadecenol to (Z)-11-hexadecenal were different from *H. virescens*. Additionally, extracts from the hybrid females contained two compounds which are produced by *H. subflexa* but not by *H. virescens*, (Z)-9-hexadecenol and (Z)-11-hexadecenyl acetate. Backcrossing females of either F_1 hybrid line to males of *H. virescens* yielded female backcross insects that produced that same pheromone blend that females of *H. virescens* produced. Extracts obtained from females from backcrosses between either of the F_1 lines and *H. subflexa* contained all of the pheromone components common to the pheromone of *H. subflexa* and the ratios of these components were similar to that of the *H. subflexa* pheromone blend. However, these backcross females also produced tetradecanal and (Z)-9-tetradecenal in amounts and ratios similar to that present in extracts obtained from *H. virescens*. Overall these results indicate that the production of pheromone by hybrid and backcross females is under polygenic by autosomal regulation. Genes from either parent species that regulate the production of the 16-carbon pheromone components do not exhibit dominant influences. However, genes from the *H. virescens* genome that regulate the production and ratio of 14-carbon aldehydes are expressed in all hybrid and backcross females.

Plans: This study has been completed and a manuscript discussing the results has been accepted for publication.

ISOLATION AND IDENTIFICATION OF PHEROMONE BIOSYNTHESIS ACTIVATING NEUROPEPTIDES FROM *HELIOTHIS VIRESCENS*

P.E.A. Teal, J.A. Meredith and J.H. Tumlinson.

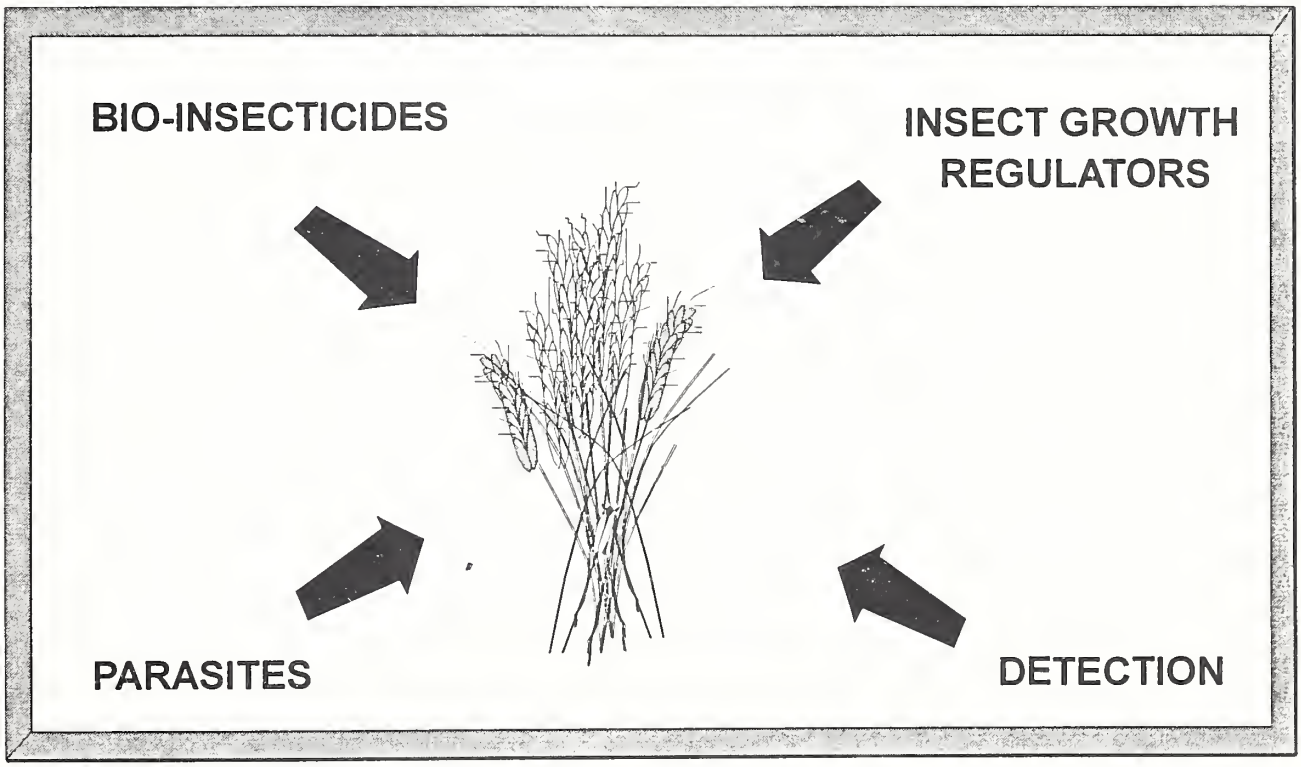
Objectives: To isolate and identify pheromonotropic neuropeptides from the nervous system of *Heliothis virescens*

Methods: The terminal abdominal ganglia and heads of females of *H. virescens* were excised and extracted with acetone prior to homogenization in aqueous 0.1% trifluroacetic acid. The extracts were bioassayed for pheromonotropic activity by injection of extracts into females of *H. virescens* during the photophase, when pheromone is not normally produced. After injection females were incubated for one hour prior to extraction of the pheromone gland and analysis of the extract to determine the amount of pheromone present by capillary gas chromatography. The extracts were subjected to cation exchange and reverse phase liquid chromatography using 5 cm X 1.5 cm columns prior to reversed phase high performance liquid chromatography (HPLC). The most potent area of activity obtained from HPLC separation of extracts from both tissues was further purified using reverse phase and inverse gradient reverse phase HPLC techniques. Fractions from each separation were subjected to pheromonotropic bioassays prior to subsequent chromatographic separations.

Results: Extracts of cephalic ganglia of females stimulated production of pheromone when injected into females during the photophase. Extracts of the terminal abdominal ganglia obtained from insects during the photophase had pheromonotropic activity but no activity was found in extracts obtained from insects during the period of pheromone production in the scotophase. Separation of the active material from both the cephalic and terminal abdominal ganglia using reversed phase HPLC showed extracts from each site contained at least three pheromonotropic peptides. Further purification of the most active compound present in extracts of both tissues showed that the same peptide was present in extracts of both the cephalic and terminal abdominal ganglia.

Plans: The structures of the pheromonotropic peptides isolated from the cephalic ganglia will be elucidated using a combination of amino acid analysis, protein sequencing and mass spectral techniques. Peptides purified from the terminal abdominal ganglia will be isolated and subjected to mass spectral analysis to determine if they are the same as those identified from the cephalic ganglia.

STORED PRODUCTS



THE EFFECTS OF JUVENOIDS ON EMBRYOGENESIS IN *PLODIA INTERPUNCTELLA*

S. Dyby and D. Silhacek

Objective: To determine the physiological basis of juvenoid toxicity during embryogenesis and early larval development of the Indian meal moth.

Methods: Freshly laid eggs were collected from the standardized laboratory culture of Indian meal moths. The eggs were carefully aged following egg laying in order to establish a chronology of developmental events that occur during embryogenesis. These events were monitored by time lapse photography, light microscopy, scanning and transmission electron microscopy, and fluorescent microscopy. Embryogenesis in the presence and absence of an effective juvenoid treatment were compared in order to identify any lesion(s) that occur in response to the treatment.

Results: A dechorionation procedure was developed so that living embryos could be observed and filmed during the course of their development into larvae. The major embryonic stages (nuclear multiplication, cellularization, gastrulation, germ band shortening, dorsal closure, embryonic molt, development of eyespots, head tanning, and the behavior of the hatching pre-larvae) were analyzed and their time of appearance recorded. Hoecht-stained embryos showed cell and segmental outlines and was useful in studying embryonic shape changes by light microscopy, even when the embryo was obscured by the serosal layer and yolk cells.

The effects of different levels (0.1 to 1.0 ppm) of juvenoids were tested by briefly dunking the eggs in an acetone solution of the juvenoid, incubating at 30° C and observing embryonic development until hatch. With both, pyroproxifen or fenoxycarb, the embryonic movements become abnormal and slow during germ band shortening. Frequently, midgut closure is prevented. Less frequently, the Malpighian tubules had abnormalities and/or portions of the tracheal system were missing. In the fenoxycarb-treated embryos embryonic molting appeared to be prevented. Some of the eggs treated with the lower juvenoid concentrations (0.01 to 0.5 ppm) hatched, giving rise to larvae having one or more developmental abnormalities. Mortality was high throughout the larval stage, usually occurring at the molts. Following pupation, some pupae had shortened wings and, in some cases, a collapsed thorax. Some supernumerary larvae were also observed following egg treatments. In contrast, first-instar larvae placed on juvenoid-treated (0.1 to 5 ppm) diets were unaffected until the final larval instar, where most continued to feed and grow, frequently undergoing one or more supernumerary larval molts. Pupation was delayed or prevented.

Plans: The two most frequently observed juvenoid-induced lesions, failures in midgut closure and molting, will be investigated more extensively to determine the biochemical basis of this lethal action.

ACOUSTICAL DETECTION OF ADULT BEETLES ASSOCIATED WITH STORED PRODUCTS

R.W. Mankin, D. Shuman, and J.A. Coffelt

Objectives: To determine whether adults of the most common pest species can be detected reliably by the Acoustic Location Fixing Insect Detector (ALFID) and estimate the minimum weight of a detectable insect.

Methods: An automated ALFID system was used to detect the presence of individual adult beetles of five species commonly found in stored grain: rice weevils, *S. oryzae*, red flour beetles, *Tribolium castaneum* (Herbst), lesser grain borers, *Rhyzopertha dominica* (L.), sawtoothed grain beetles, *Oryzaephilus surinamensis* (L.) and rusty grain beetles, *Cryptolestes ferrugineus* (Stephens). The system consists of a 1-kg sample container (76 x 5 x 4 cm) with a linear array of 16 (numbered 0-15) piezoelectric sensors mounted in one wall. A 16-channel electronic circuit board positioned on an adjacent wall amplifies and filters the analog output of each sensor. Amplitude threshold detection is used to delineate the arrival time of any acoustical signal. The unit is cabled to a custom logic circuit interfaced with a digital input/output board installed in a microcomputer. Custom software records the location and timing of each above-threshold sound.

Results and Plans: Sounds produced by individuals of the three largest species: red flour beetle, rice weevil, and lesser grain borer, were detected within 1 minute in 100% of the tests (Table 1). Individual adults of the smaller sawtoothed grain beetle, were detected in 60% of the tests. Numbers of sounds produced by individual adults of the rusty grain beetle, the smallest species tested, were significantly greater than the noise background in 23% of the tests. The results indicate that ALFID can easily detect active adult insects that weigh > 0.6 mg in 1 kg samples of grain.

Because insects are not necessarily detected when they are inactive, efforts are in progress to increase the likelihood that insects will be active during the time when they are sampled. Experiments are continuing with the use of heat treatments to stimulate activity.

Table 1. Percentage of individual adults of different species detected by ALFID ± Standard Error (SEM) and average number of sounds/min ± SEM.

Species	Percentage ± SEM of detected individuals	Mean ± SEM Sounds/min[1]
Uninfested	-	2.7 ± 0.4 a
C. ferrugineus	23.3 ± 6.7	4.6 ± 0.6 a
O. surinamensis	60.0 ± 8.2	11.6 ± 3.4 b
R. dominica	100.	212.2 ± 48.6 c
S. oryzae	100.	529.9 ± 76.4 d
T. castaneum	100.	675.1 ± 131.5 d

[1]Means followed by the same letter are not significantly different (Tukey's Studentized range test [SAS Institute, 1985]).

GROWTH AND DEVELOPMENT OF THE INDIANMEAL MOTH: ACTION OF TEBUFENOZIDE, A NON-STEROIDAL ECDYSTEROID AGONIST

H. Oberlander, D.L. Silhacek, C.E. Leach and S. Parisek

Objective: To determine the action of tebufenozide on growth and molting in the Indianmeal moth as a basis for control of this stored product pest with insect growth regulators that mimic ecdysteroids.

Methods: In vitro procedures: Mesothoracic wing imaginal discs were dissected from late last instar larval Indianmeal Moths under sterile conditions and cultured in modified Grace's insect tissue culture medium in 35mm diameter plastic tissue culture dishes. Incorporation and uptake of ^{14}C-GlcNAc by the cultured wing discs was measured after application of tebufenozide (RH5992) and compared with the effects of 20-hydroxyecdysone. In vivo procedures: Indianmeal Moths were reared on a cereal diet and larvae that weighted approximately 3 mg were selected for tests. Larval diet was treated with various concentrations of tebufenozide, methoprene (a juvenile hormone mimic), or a mixture of the two. Insect weight and developmental stage were checked during a one month period for each treatment.

Results: After a 48 hour treatment with tebufenozide, both amino-sugar uptake and incorporation were stimulated in the cultured wing discs. The threshold for activity was 0.1 μM for incorporation of the chitin precursor, and 0.01 μM for increased uptake. This high activity at low concentrations was seen as well in the tests with intact larvae, in which tebufenozide was active at 1/5th the concentration of a related ecdysteroid mimic, RH5849. A review of both basic and applied research was published in which the potential of ecdysteroid mimics (dibenzoyl hydrazines) for practical control of agricultural pests was assessed.

Plans: There will be increased focus on the potential for tebufenozide as an effective control agent for stored product lepidoptera. In addition, as an ecdysteroid mimic, tebufenozide provides opportunities for examining the effectiveness of juvenile hormone mimics in both tissue culture and in vivo test systems. Together these studies with insect growth regulators comprise part of a larger study on alternatives to methyl bromide in protecting stored commodities from insect attack.

INHIBITION OF PROLIFERATION IN AN INSECT CELL LINE

H. Oberlander, P. J. Hatt[1] and P. Porcheron[1]

Objective: To determine whether tebufenozide, an ecdysteroid agonist, inhibits cellular proliferation in an established cell line.

Methods: Cellular proliferation was assessed through the non-radioactive quantification of cleavage of a tetrazolium salt (MTT) by dehydrogenase activity in active mitochondria. The resulting formazan crystals are solubilized and the color of the solution is quantified with an ELISA reader. This method, adapted to use on insect cells by P. J. Hatt and P. Porcheron, provides a convenient, rapid and quantitative measure of proliferation without the use of radioisotopes. A cell line, IAL-PID2, established from imaginal discs of the Indianmeal Moth was used in the assays.

Results: Initial experiments with the MTT method confirmed that 20-hydroxyecdysone inhibited proliferation in the cell line in a dose dependent manner. Moreover, tebufenozide (RH5992) was effective in inhibiting proliferation in the cell line at concentrations of 1-^{7}M or higher. The dose-response relationship of this inhibitory activity was virtually indistinguishable from the effects on 20-hydroxyecdysone.

Plans: This work was conducted while H. Oberlander was a visiting scientist in the laboratory of Prof. Porcheron. The non-radioactive method for assessing cellular proliferation may have application to other tests of insect growth regulators in tissue culture.

[1] Département de Biologie, Ecole Normale Supérieure, Paris, France

DISTRIBUTION OF TAN AND WHITE FAT BODY HISTOTYPES IN MOTHS OF THE PYRALIDAE FAMILY.

P.D. Shirk and G. Zimowska-Handler

Objective: To determine the distribution of the tan and white fat body histotypes, which was discovered in the Indianmeal moth, *Plodia interpunctella*, in larvae of moths from the Pyralidae family and correlate the presence of the tan fat body histotype with the ability of the larvae to consume seeds as a primary food source.

Methods: The fat bodies of five moths from the four subfamilies of the Pyralidae, the melon worm *Diaphania hyalinata* (L) (Pyraustinae); the meal moth, *Pyralis farinalis* (L) (Pyralinae); the greater wax moth, *Galleria mellonella* (L) (Galleriinae); navel orange worm, *Amyelois transitella* (Oliver) (Phycitinae); and the Indianmeal moth, *Plodia interpunctella* (Hübner) (Phycitinae); and one moth from the Gelechiidae family, the Angoumois grain moth, *Sitotroga cerealella* (Oliver) (which can feed on moist grain), were examined using transmission electron microscopy to assess the histotypes present during the larval stages. Morphometric measurements were made on the number and size of the mitochondria in the fat body tissues of each of the species.

Results: The tan fat body and white fat body histotypes were found in *A. transitella* and *P. interpunctella*, both members of the subfamily, Phycitinae. Only the white fat body histotype was observed in *D. hyalinata* (Pyraustinae) and *P. farinalis* (Pyralinae) of the Pyralidae family and in *S. cerealella* of the Gelechiidae family. However, although the tan fat body histotype was observed in *G. mellonella* (Galleriinae), it was only present in the first six larval instars but not in the last larval instar. The mitochondria of the tan fat body histotype were uniformly larger and more numerous than the mitochondria in white fat body. This data suggests that the tan fat body histotype is correlated with the taxonomic division of the Pyralidae family. In addition, it is the members of the Phycitinae subfamily, which are primarily seed feeders, that possess the two fat body histotypes. Therefore, the presence of the tan fat body histotype may be necessary for their ability to feed on dried commodities and presents a potential target for effecting control of these stored-product insects.

Plans: The role of tan fat body in the production of metabolic water will be examined. In addition, the toxicity of pyroles that uncouple mitochondria respiration will be assessed as an alternative chemical treatment for the control of stored-product pest insects.

ACOUSTIC LOCATION FIXING INSECT DETECTOR (ALFID)

D. Shuman, J. Coffelt, and R. Mankin

Objective: To develop and evaluate an economically feasible inspection method to automatically identify the number of live insects, including internal-feeding larvae, in a grain sample. Present visual inspection methods can only detect adults because the larvae develop inside grain kernels and thus comprise a hidden infestation.

Methods: An internal-feeding larva is normally not visible, but its presence can be revealed by detection of sounds it produces as it feeds within a grain kernel. Some degree of quantification of infestation can be statistically achieved by its correlation with total acoustic activity. However, this method is not sufficiently accurate for sample grading. ALFID quantifies infestation by ascertaining the number of acoustic source locations in a grain sample. The system incorporates an array of acoustic sensors mounted in a grain sample container. The location of a particular sound's source can be determined by identifying the different sensors in the array that receive the sound, and the time intervals between those receptions. Multiple sounds originating from the same location indicates the presence of an insect at that location.

Results: The prototype of the ALFID system that demonstrated feasibility of the operational concept for quantifying heretofore generally undetectable infestations of internal-feeding larvae as well as adult beetles was redesigned in an effort to improve its performance. The new grain container is a tube with two opposing arrays of 8 piezoelectric microphones along its length. A CRADA has been established to concurrently investigate applying a new acoustic sensor technology using fluidic amplification to reduce the electronic noise problems associated with detecting extremely low level larval sounds in the ALFID system. Acoustic signals from the sensor arrays are acquired by a customized A/D board in a PC computer and processed using cross-correlation and other time domain analyses to ascertain the time intervals (relative delays) between sensor output signals and reduce data artifacts by culling. The resulting spreadsheet summarizes all pertinent cross-correlation results for all detected sounds in a single grain sample test and initial tests with infested grain kernels indicate better than an order of magnitude improvement in localization resolution compared with the original prototype. Operation of the system in a noisy field environment is addressed by the use of acquisition-inhibiting ambient noise detectors mounted on the outside of the grain container which is then housed in a multi-layered sound attenuation box. The sound attenuation box can be rotated to different positions to facilitate the filling and emptying of the grain container without its being removed from the box and initial testing showed an average sound attenuation of 77 dB over the pertinent frequency range (1-10 kHz).

Plans: A cluster analysis algorithm to reduce the cross-correlation spreadsheet results to numbers of insects in samples is being developed. ALFID will then be laboratory and field tested. Potential future plans include the incorporation of fluidic sensor technology and neural network grouping of sounds. Behavioral research on factors affecting insect acoustic activity will be continued to increase the probability of detection during a sample test period. A patent on the ALFID system is pending and licensing for its commercialization will be pursued.

ELECTRONIC GRAIN PROBE INSECT COUNTER (EGPIC)

D. Shuman and J. Coffelt

Objective: To develop and evaluate a grain probe that counts insects electronically as they fall through. Commercial grain probe traps presently available are effective in monitoring low infestation levels in stored grain. However, ascertaining the trap insect count is labor intensive involving removal of the trap from the grain, visual inspection of the contents, and re-insertion of the trap. There is also the tradeoff between trap inspection frequency, timely discernment of infestation, and cost. EGPIC would eliminate these concerns by providing remote real-time data.

Methods: A commercial grain probe trap was modified by incorporating a custom sensor head with infrared electronics to count insects as they fall through. A versatile modular design allows for multiple system implementations to address different applications. For example, a system suitable for small storage facilities was constructed that connects the grain probe to a control box that is mounted on the outside of a grain bin. The control box has an LED insect count display, a power interrupt indicator, and self-test and reset controls. A computer-based system was developed that creates a data base of the time-stamped insect counts to be used for trend analysis, input to an expert management system, and automated control measures. It uses the printer port to interface with up to 8 probes (an installed digital I/O computer board could interface with up to 95 probes) for instantaneous (interrupt driven) probe activity updates. Another version, suitable for large storage facilities with thousands of probes miles away from a central computer, stores the insect count of each probe in its own digital register that can be polled through the serial port of the computer via a transmission network (SMARTS) designed for this purpose.

Results: Laboratory testing with infested grain showed >95% accuracy for the full range of pertinent insect species sizes. Field tests in a flat storage of corn in Wisconsin found that EGPIC overestimated the actual numbers of insects passing through the probes. Grain particles and dust that passed by the sensors, and the movement of minute insects and mites back and forth over the sensors, contributed to increased counts. Additionally, beam paths became obscured with accumulated dust as sampling time progressed. Nevertheless, regression analysis revealed that EGPIC counts could reliably predict (R^2=0.897) numbers of insects entering probes across a range of insect densities. Use of a different probe body with upward slanted holes resulted in fewer grain particles entering the probe and being counted. The sensor head has been redesigned to reduce the possibility of insects crawling and dust accumulating in the vicinity of the infrared beam. A patent is pending on the EGPIC system.

Plans: The redesigned sensor head will be evaluated in the laboratory and then field tested in a Williston, FL commercial wheat bin. Research will be conducted on the interpretation and utilization of the realtime data available with the EGPIC system. Enhancements of EGPIC that allow for species differentiation will be investigated.

SERIAL MULTIPLEXING ADDRESSABLE REGISTER TRANSMISSION SYSTEM (SMARTS)

D. Shuman

Objective: To develop an economically attractive data transmission facility suitable for data collection by a single PC type computer from a large number of remote sensor locations distributed throughout a large grain storage facility. This system could be used in conjunction with the EGPIC System and with the acoustic sensors mounted on cables for monitoring insect infestation.

Methods: The original design of the SMARTS facility was modified to halve the amount of wire needed by time sharing single twisted wire pairs for transmitting and receiving. It is capable of automatically sequencing through any programmed order of remote locations or manually selecting any individual remote location. The presently implemented 3 level version could read up to 512 one byte digital registers, and a proposed 4 level version could read up to 4096 such registers. Using RS-422 protocol, the computer could be located up to 3 miles away from the furthest register.

Results: The original prototype constructed in the laboratory demonstrated system feasibility. Based on a SMARTS Patent Disclosure, the ARS Patent Committee decided to pursue a patenting.

Plans: The time sharing feature will be tested and the system software will be expanded to provide a user interface. The system will be tested using long transmission lines. A patent application will be prepared and submitted to the U.S. Patent Office.

CONSTRUCTION OF CABLES AND IMPLEMENTATION OF THE PILOT TEST "AUTOMATION OF STORED GRAIN INSECT POPULATION MONITORING WITH ACOUSTICAL SENSORS"

D. Shuman and D. Hagstrum

Objective: To design, construct, and test cables with attached piezoelectric acoustic sensors to be mounted in grain storage bins and to implement data acquisition systems at test sites.

Methods: Six cables with 12 sensors attached to each cable and 24 cables with 20 sensors attached to each cable were built and shipped to the ARS U.S. Grain Marketing Research Laboratory in Manhattan, KS. The cables were installed in the pilot test farm bins and attached to a data acquisition system consisting of an analog multiplexer, an amplifier, a bandpass filter, and a peak counter, all interfaced with a PC type computer.

Results: The acoustical system detected insect infestation in wheat bins 16 to 31 days earlier than with visual inspection of grain samples. The frequency of sound detection was correlated with insect densities in grain samples over the range of 0.5 to 7.5 insects per kilogram of grain. A manufacturer of thermocouple cables for grain storage facilities has expressed interest in commercializing an acoustic cables insect monitoring system and is cooperatively investigating manufacturing feasibility of the cables.

Plans: The pilot test was completed. Prototype manufactured cables will be constructed and evaluated. Custom electronics will be designed to allow the data gathered by the acoustic sensors to be locally digitized and accumulated to be transmitted to the computer via the SMARTS data transmission facility.

A RAPID METHOD FOR ASSESSING THE NUMBER OF WEEVILS INFESTING STORED GRAIN

D. Silhacek, J. Baker[1] and J. Throne[1]

Objective: To develop a method for the rapid, simple determination of the number of weevils infesting samples of stored grain.

Methods: We will isolate and characterize proteins that constitute the egg plug and raise antibodies to one or more of the egg plug proteins. We will then determine the feasibility of using the immune reaction in a bioassay; e.g. conjugating with x-ray opaque component to visualize plugs or by developing an assay, such as the ELISA or ELIFA, to quantitate egg plug protein in a grain sample. The number of egg plugs or the amount of egg plug protein located in a fixed number of sample kernels of grain will be equated to the level of infestation.

Results: A method has been developed for quantitating total weevil egg plug protein in samples of shelled corn. The current method does not distinguish between the granary weevil, maize weevil and rice weevil. The procedure will detect one egg plug in a 100 kernel sample of corn. Studies on estimating the levels of weevils in samples of stored corn in the field are now underway at the Manhattan USDA Laboratory. The authors have applied for a patent on this method of weevil detection and are currently writing a manuscript that describes the procedure.

Plans: We will complete our evaluation of the methodology for its accuracy in equating egg plug protein levels to the levels of infestation. If this appears to be a viable strategy, we will proceed with refining the procedures based on this technology so as to improve sensitivity and accelerate the analyses of grain samples.

[1] US Grain Marketing Research Lab, USDA, Manhattan, Kansas.

THE POTENTIAL USE OF JUVENOIDS FOR PROTECTING STORED PRODUCTS FROM INSECT PESTS

D. Silhacek and C. Murphy

Objective: To determine if treatments with the juvenoids, fenoxycarb and pyriproxyfen, could protect commodities from insect damage during storage.

Methods: Freshly laid eggs were collected from standard laboratory cultures of P. interpunctella. Initially, eggs were treated by immersing them in acetone solutions of the test juvenoids and immediately transferring to blotting paper using a cut-tip pipette. The treated eggs were placed on a small amount of diet and held at standard rearing conditions. The number of insects hatching and growing to maturity were recorded and any anomalies in development were monitored. With these procedures, we will determine the most susceptible stages in the insects development. Methods of treating stored commodities with juvenoids will be developed that will intervene during the susceptible stage of the insect's development.

Results: The immersion tests revealed that freshly laid eggs succumbed before hatching when briefly dunked in acetone solutions of either juvenoid at levels as low as 1.0 ppm. Only the first 18 hours of embryogenesis was susceptible to this treatment; applications to eggs over 20 hours old were ineffective, even a higher juvenoid concentrations. Lower juvenoid dosages applied during the first 18 hours, delayed mortality until the first or second larval stadium. Our current studies are examining various methods of delivering juvenoids to eggs during this susceptible period. We have found that freshly laid eggs fail to hatch when placed on juvenoid-treated surfaces. This approach may be of considerable value for protecting packaged commodities. Taking these observations a step further, we have found that egg hatch could also be prevented by treating the adult female moth with juvenoid. The treatment could be either a topical application or through contact with a juvenoid treated surface. We speculate that juvenoid treatment of containers containing processed commodities may be doubly effective because of its contact activity, first on the adult female and then on the eggs. Thus, one reaches the conclusion that an effective juvenoid treatment interferes with some fundamental process during a very early stage of embryogenesis. The observations that juvenoid agonists can act so effectively at low concentrations and with such a brief exposure during embryogenesis suggests that treating stored commodities with juvenoids may provide an effective alternative to methyl bromide treatments.

Plans: We will pursue our current investigations on developing more effective protocols for protecting stored commodities with juvenoids. Using our current technology, we will examine the effectiveness of juvenoid-treated packaging in protecting packaged foods from insect damage during storage.

PRODUCTION AND RELEASE OF SEX PHEROMONE BY FEMALES OF *PLODIA INTERPUNCTELLA*.

P.E.A. Teal, R.R. Heath, B.D. Dueben, J.A. Coffelt, and K.W. Vick

Objectives: To determine the biosynthetic and endogenous mechanisms regulating pheromone production by females of *Plodia interpunctella*.

Methods: The pheromone glands of females were excised and extracted in hexane, containing internal standards, at the time of adult emergence and at 2 h intervals thereafter to determine the age at which females began to produce pheromone and the diel periodicity of pheromone production. Studies on the endogenous regulation of pheromone production were conducted using females decapitated at the time of emergence. Four h after decapitation females were injected either with extracts of the heads of females or with saline and incubated for various times prior to excision and extraction of the pheromone glands. Volatile pheromone components released by calling females were collected during consecutive 2 h periods during the 2nd scotophase after adult emergence from groups of 20 females. Volatiles, collected on Super Q filters, were eluted by washing the filters with methylene chloride. Studies on the terminal steps in pheromone biosynthesis were conducted by incubating isolated pheromone glands with alcohol or acetate precursors for various periods and then extracting the tissue and incubation media with hexane. Chemical analyses were conducted using capillary gas chromatography (GC) with both polar and apolar capillary columns and by GC-mass spectroscopy.

Results: Extracts of sex pheromone glands obtained from females contained pheromone 4 h prior to the first scotophase after adult emergence. The amount of pheromone increased during the first 4 h of the scotophase and then declined to low levels during the subsequent photophase. Decapitation of females immediately after emergence, inhibited production of pheromone during the subsequent 48 h. Injection of extracts of the heads of 1 day-old females into decapitated females stimulated production of both (Z,E)-9,12-tetradecadien-1-ol acetate (Z9E12-14:AC) and (Z,E)-9,12-tetradecadien-1-ol (Z9E12-14:OH) as well as production of (Z,E)-9,12-tetradecadienal (Z9E12-14:AL). This aldehyde was subsequently identified from extracts of pheromone glands obtained from naturally calling females as well as from volatiles emitted by calling females. Studies on the terminal steps in biosynthesis of the pheromone showed that Z9E12-14:OH was produced from Z9E12-14:AC and that Z9E12-14:AL was produced from the alcohol via the action of an oxidase(s).

Plans: This study has been completed and a manuscript discussing the results has been accepted for publication.

LABORATORY PUBLICATIONS

INSECT ATTRACTANTS, BEHAVIOR, AND BASIC BIOLOGY RESEARCH LABORATORY

LABORATORY PUBLICATIONS LIST

Reprints may be requested by number
Address inquiries to:

H. Oberlander, Laboratory Director
USDA/ARS, P.O. Box 14565, Gainesville, Florida 32604 USA

94.1 **Sivinski, J.M., N. Epsky, and R.R. Heath. 1994.** Pheromone Deposition on Leaf Territories by Male Caribbean Fruit flies, *Anastrepha suspensa* (Loew) (Diptera:Tephritidae). J. Insect Behav. 7(1):43-51.

94.2 **Silhacek, D.L., S.G. Miller, and C.L. Murphy. 1994.** Purification and Characterization of a Flavin-Binding Storage Protein From the Hemolymph of *Galleria mellonella*. Arch. Insect Biochem. & Physiol. 25:55-72.

94.3 **Mikolajczyk, P., H. Oberlander, D.L. Silhacek, I. Ishaaya, and E. Shaaya. 1994.** Chitin Synthsis in *Spodoptera frugiperda* Wing Imaginal Discs: I. Chlorfluazuron, and Teflubenzuron Inhibit Incorporation But Not Uptake of {^{14}C]N-acetyl-D-glucosamine. Arch. Insect Biochem. & Physiol. 25:245-258.

94.4 **Zimowska, G., P.D. Shirk, D.L. Silhacek, and E. Shaaya. 1994.** Yolk Sphere Formation is Initiated in Oocytes Before Development of Patency in Follicles of the Moth, *Plodia interpunctella*. Roux's Arch. Dev. Biol. 203:215-226.

94.5 **Tumlinson, J.H., E.R. Mitchell, R.E. Doolittle, and D.M. Jackson. 1994.** Field Tests of Synthetic *Manduca sexta* Sex Pheromone. J. Chem. Ecol. 20(3):579-591.

94.6 **Heath, R.R., and A. Manukian. 1994.** An Automated System For Use in Collecting Volatile Chemicals Released From Plants. J. Chem. Ecol. 20(3):593-608.

94.7 **Oberlander H. and S.M. Ferkovich. 1994.** Physiological and Developmental Capacities of Insect Cell Lines. In: Insect Cell Biotechnology. CRC Press, Boca Raton, pp 127-140.

94.8 **Ferkovich S.M., H. Oberlander, C. Dillard, and E. Leach. 1994.** Embryonic Development of an Endoparasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae) in Cell Line-Conditioned Media. In Vitro Cell. Dev. Biol. 30A:279-282.

94.9 **Oland, L.A. and H. Oberlander. 1994.** Growth and Interactions of Cells From the Insect Nervous System *In vitro*. In: Insect Cell Biotechnology. CRC Press, Boca Raton, pp. 104-124.

94.10 **Sanders, B.M., N. Julie, T.G. Douglass, and S. Miller. 1994.** Heat-Inducible Proteins That React with Antibodies to Chaperonin are Localized in the Nucleus of a Fish Cell Line. J. Biochem. 20:21-25.

94.11 **Landolt, P.J. 1994.** Fruit of *Morrenia odorata* (Asclepiadaceae) as a Host For the Papaya Fruit Fly, *Toxotrypana curvicauda* (Diptera: Tephritidae). Fla Entomol. 77(2):287-288.

94.12 **Mitchell, E.R. and J.H. Tumlinson. 1994.** Response of *Spodoptera exigua* and *S. eridania* (Lepidoptera: Noctuidae) Males to Synthetic Pheromone and *S. Exigua* Females. Fla Entomol. 77(2):237-247.

94.13 **Abernathy, R.L., P.E.A. Teal, and J.H. Tumlinson. 1994.** Age and Crowding Affects the Amount of Sex Pheromone and the Oviposition Rates of Virgin and Mated Females of *Helicoverpa zea* (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 87(3):350-354.

94.14 **Greany, P.D. 1994.** Plant Host Status and Natural Resistance. Paull, R.E. and Armstrong, J.W. (eds.). 1994. Insect Pests and Fresh Horiculture Products: Treatments and Responses. CAB International, Wallingford, UK.

94.15 **Mankin, R.W. 1994.** Acoustical Detection of Aedes Taeniorhynchus Swarms and Emergence Exoduses In Remote Salt Marshes. J. Am. Mosq. Control Assoc. 10(2):302-308.

94.16 **Greany, P.D., R.E. McDonald, W.J. Schroeder, P.E. Shaw, M. Aluja and A. Malavasi. 1994.** Use of Gibberellic Acid To Reduce Citrus Fruit Susceptibility to Fruit Flies. ACS Symp. No. 557:40-48

94.17 **Heath, R.R., N.D. Epsky, S. Bloem, K. Bloem, F. Acajabon, A. Guzman, and D. Chambers. 1994.** pH Effect on the Attractiveness of a Corn Hydrolysate to the Mediterranean Fruit Fly and Several *Anastrepa* Species (Diptera:Tephritide). J. Econ. Entomol. 87(4):1008-1013.

94.18 **Teal, P.E.A. and R.J. Nachman. 1994.** Seperation of Neuropeptides by Inverse Gradient Reversed Phased Liquid Chromatography on a Bonded Phenyl Support. Analyt. Biochem. 221:422-425.

94.19 **Heath R.R., N.D. Epsky, B.D. Dueben, A. Guzman, and L.E. Andrade. 1994.** Gamma Radiation Effect on Production of Pheromonal Components of Male Mediterranean Fruit Flies (Diptera: Tephritidae) J. Econ. Entomol. 87(4):904-909.

94.20 **Epsky N.D., R.R. Heath, T.C. Holler, D.L. Harris, and T. Mullins. 1994.** Corn Steepwater as Protein Bait for *Anastrepha suspensa* (Diptera: Tephritidae). Environ. Entomol. 23(4):827-831.

94.21 **Tingle, F.C., E.R. Mitchell, and J.R. Mclaughin. 1994.** Lepidopterous Pests of Cotton and their Parasitoids in a Double-Cropping Environment. Fla. Entomol. 77(3):335-341.

94.22 **Landolt P.J. 1994.** Mating Frequency of the Papaya Fruit Fly (Diptera: Tephritidae) With and Without Host Fruit. Fla. Entomol. 77(3):306-312

94.23 **Zimowska G., P. Mikolajczyk, D.L. Silhacek, and H. Oberlander. 1994.** Chitin Synthesis in *Spodoptera frugiperda* Wing Imaginal Discs. II. Selective Action of Chlorfluazuron on Wheat Germ Agglutinin Binding and Cuticle Ultrastructure. Arch. Insect. Biochem. & Physiol. 27:89-108

94.24 **Boucias, D.G., J. Pendland, C.-C. Lopez-Lastra, and J. Tumlinson. 1994.** In vitro development of *Nomuraea Rileyi*: Regulation of the Hyphal Body - Mycelia Transition. VIth Intl. Colloq. Invert. Pathol. & Microbial Control. XXVII Ann. Mtg Soc. Invert. Pathol. Montpellier, France, 28 Aug - 2 Sept. 1994. Abstract.

94.25 **Shirk, P.D. and K.L. Ogren. 1994.** "Reduced Appendage": A Mutation Affecting Development of Pupal and Adult Appendages of Moth, *Plodia interpunctella.* Arch. Insect Biochem. Physiol. 27:179-191.

94.26 **Oland L.A. and H. Oberlander. 1994.** Factors that Influence the development of Cultured Neurons from the Brain of the Moth *Manduca Sexta*. In Vitro Cell Dev. Biol. 30a:709-716.

94.27 **Hatt P.J., M. Moriniere, H. Oberlander, and P. Porcheron. 1994.** Roles for Insulin and Ecdysteroids in Differentiation of an Insect Cell Line of Epidermal Origin. In Vitro Cell Dev. Biol. 30a:709-716.

94.28 **Mitchell E.R. and J.A. Leach. 1994.** A Yellow Mutant *Heliothis virescens* (Lepidoptera Noctuidae). J. Entomol. Sci. 29(4):509-512.

94.29 **McLaughlin J.R., E.R. Mitchell, and P. Kirsch. 1994.** Mating Disruption of Diamondback Moth (Lepidoptera: Plutellidae) in Cabbage: Reduction of Mating and Suppression of Larval Populations. J. Econ. Entomol. 87(5):1198-1204.

94.30 **Landolt P.J., R.R. Heath, J.G. Millar, K.M. Davis-Hernandez, B.D. Dueben, and K.E. Ward. 1994.** Effects of Host Plant, *Gossypium hirsutum* L., On Sexual Attraction of Cabbage Looper Moths, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). J. Chem. Ecol. 20(11):2959-2974.

94.31 **McCall P.J., T.C.J. Turlings, J. Loughrin, A.T. Proveaux, and J.H. Tumlinson. 1994.** Herbivore-Induced Volatile Emissions from Cotton *(Gossypium hirsutum L.)* Seedlings. J. Chem. Ecol. 20(12):3039-3050.

94.32 **Loughrin J.H., A. Manukian, R.R. Heath, T.C.J. Turlings, and J.H. Tumlinson. 1994.** Diurnal Cycle of Emission of Induced Volatile Terpenoids by Herbivore-Injured Cotton Plants. Proc. Nat. Acad. Sci. 91:11836-11840.

94.33 **Sivinski, J.M., C.O. Calkins, and R. Baranowski. 1994.** A Container for Eclosion and Holding Adult Insects Prior to Mass Release. Fla. Entomol. 77(4):513-514.

94.34 **Ferkovich, S.M. and D. Blumberg. 1994.** Acceptance of Six Atypical Host Species for Ovipostion by *Microplitis Croceipes* (Hymenoptera: Braconidae). Israel J. Entomol. 28:123-131